Studies on the genetics of ovine behaviour in a Western Cape resource flock aimed at improving animal welfare

by

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Summary

Data were collected on a Merino resource flock on the behaviour of ewes and lambs from 1993 to 2002. This flock was divergently selected from the same base population since 1986 for the number of lambs weaned per mating (NLW). Selection resulted in two selection lines, namely the High line (H line, upward selection for NLW) and the Low line (L line, downward selection for NLW). Additionally, the effect of these behaviours and selection lines were reported for NLW and lamb survival, as a key welfare indicator trait. Data from arena behaviour on Merino weaners were also collected over a 15-year period using the same resource population divergently selected for NLW. Ram lambs, progeny of mature ewes and singles were heavier at birth than ewe lambs, progeny of young and old ewes and multiples respectively. H line lambs had shorter lengths of parturition than L line lambs, where this difference was also found in H line and L line ewes. The effects of dam age were significant for the latency from birth to suckling as it improved with ewe age, and in both ewe and lamb studies H line ewes had better maternal cooperation scores than L line ewes. Maternal cooperation also had a significant effect on the latency for the lamb to progress from birth to suckling and maternal cooperation score improved with age in ewes. H line ewes had an improved NLW compared to L line ewes while H line lambs had a better survival. For production traits, H line lambs derived from embryo transplants were heavier at yearling age, had a lower fibre diameter, less wrinkles at the neck, body, breech and overall when compared to L line lambs. Survival of lambs reared by surrogate dams was higher in the H line than in the L line. Single-trait direct heritability estimates (h^2) in lambs were 0.15 for birth weight, 0.07 for lamb survival, 0.06 for length of parturition, 0.12 for latency from birth to suckling and 0.00 for maternal cooperation score. Direct heritability estimates, assessed as trait of the ewe, were 0.04 for NLW, 0.17 for length of parturition, 0.07 for maternal cooperation score and 0.20 for the interval ewes remained on or near their lambing sites. Genetic trends suggested divergence between the lines for breeding values for length of parturition, suggesting that parturitions became shorter in the H line and longer in the L line. The results and parameter estimates obtained, suggested that selection for improved NLW would yield desirable outcomes for ewe behavioural traits. Maternal permanent environment variance ratios (c^2) were 0.07 for lamb survival and 0.17 for maternal cooperation score. Survival of the lamb was favourably correlated to the length of parturition and maternal behaviour score on the genetic level. It was evident that neonatal behaviour was, to an extent, under genetic control in paddock-reared sheep. In the arena test, animals from the H line approached closer to stationary human seated between the test animal and its flock mates and had fewer urination and defecation events than their L line contemporaries but travelled longer distances in the arena. The distance the lambs maintained from the human operator (0.08), urinating events (0.13), and defecating events (0.04) were all lowly heritable. However, the number of lines crossed (0.22) and the number of bleats (0.35) were moderately to highly heritable. Selection for NLW and certain neonatal and perinatal ewe behaviours would benefit the welfare of ewes and lambs. From the arena test it was also evident as H line lambs experienced lower levels of stress in the contrived area environment.

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Opsomming

Data van ‘n Merino hulpbronkudde oor die gedrag van ooie en lammers is vanaf 1993 tot en met 2002 versamel. Die kudde was uiteenlopend geselekteer vanaf dieselfde basispopulasie sedert 1986 vir getal lammers gespeen per paring (NLW). Seleksie het gelei to twee seleksielyne, naamlik die Hoë lyn (H lyn, opwaartse seleksie vir NLW) en die Lae lyn (L lyn, afwaartse seleksie vir NLW). Daarbenewens is die effek van die gedrag en seleksielyn vir NLW en lamoorlewing as ‘n noodsaaklike welsynseienskap gerapporteer. Data van die arenagedrag op Merino speenlammers is ook oor 15 jaar versamel deur dieselfde basispopulasie te gebruik. Ramlammers, nageslag van volwasse ooie en enkelinge was swaarder by geboorte as ooi lammers, nageslag van jong en ouer ooie en meerlinge. H lynlammers het ‘n korter duur van geboorte gehad as L lynlammers. Die verskil het ook voorgekom in die parturisies van H lyn- en L lynooie. Die effek van die ouderdom van die ooi was betekenisvol vir die tyd vanaf geboorte tot en met suip en dit het verbeter met ooiouderdom. H lynooie het in beide die ooi- en lamstudies beter met hulle lames se eerste suippogings saamgewerk as L lynooie. Maternale samewerkingstellings het ook ‘n betekenisvolle effek gehad op die tyd wat dit vir die lam gevat om van geboorte tot suip te vorder. Maternale samewerkingtellings het verbeter met ooi-ouderdom. H lynooie het ‘n beter NLW gehad as L lynooie, terwyl H lynlammers beter oorleef het het. H lynlammers met embrio-oorplasing geproduseer was swaarder in vergelyking met die L lyn, met ‘n laer veseldikte, minder plooie op die nek, lyf, broek, en algeheel in vergelyking met die L lyn. Oorlewing van lammers wat deur surogaat ooie grootgemaak is, was hoër in die H lyn as in die L lyn. Enkel eienskap direkte oorerflikheidberamings (h^2) in lammers was 0.15 vir geboorte gewig, 0.07 vir lam oorlewing, 0.06 vir die duur van geboorte, 0.12 vir die tyd vanaf geboorte tot suig en 0.00 vir maternale samewerkingstellings. Direkte oorerflikheid beramings, as ‘n eienskap van die ooi ontleed, was 0.04 vir NLW, 0.17 vir duur van geboorte, 0.07 vir Maternale sameweking en 0.20 vir die tyd wat die ooi naby of op die lamplek gebly het. Genetiese tendense dui op teenoorgestelde genetiese tendense tussen die lyne. Teel waardes vir duur van geboorte het aangedui dat geboortes korter in die H lyn geword het en langer geword het in die L lyn. Die verkreë resultate en parameterberamings dui daarop dat seleksie vir ‘n verbeterde NLW gunstige uitkomste lewer vir ooi- en lamgedrag. Lamoorlewing was op die genetiese vlak gunstig gekorreleer met die duur van geboorte en maternale samewerking. Dit was duidelik dat neonatale gedrag tot ‘n mate onder genetiese beheer was in vryweidende skape. In die arenatoets het die lammers van die H lyn nader gekom aan ‘n sittende persoon tussen die dier en sy/haar tropmaats, en het ook minder geurineer en gemis as tydgenote in die L lyn, maar het langer afstande in die arena afgelê. Die demiddelde afstand van die lammers vanaf die persoon (0.08), urineringsvoorvalle (0.13) en ontlastingsvoorvalle (0.04) was almal laag oorerflik. Alhoewel, die aantal lyne gekruis (0.22) en die aantal keer geblêr (0.35) was matig tot hoog oorerflik. Die gunstige effek van seleksie vir NLW op neonatale- en perinatale ooi- en lamgedrag sal die welsyn van die ooi en lammers bevoordeel. H lynlammers het laer vlakke van stres in die aangepaste omgewing ervaar.

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Chapter 1
General Introduction

Lamb mortality is a serious economic and welfare issue (Mellor & Stafford, 2004). Research during the past 50 years, which was driven primarily by economic considerations, has shed light on most of the causes and means of preventing neonatal mortality of production animals, including practical means of decreasing its incidence. Perinatal mortality is defined as the death of the neonate from before birth to 3-7 days of age. In lambs, the major share of deaths occurred within the first 3 days (Cloete, 1992; Nowak *et al.*, 2000; Sawalha *et al.,* 2007; Brien *et al.*, 2010). Neonatal mortality rates in lambs have been kept between 15 - 25% world-wide (Hight & Jury, 1970; Alexander, 1984; Nowak & Poindron, 2006), but can be farm dependent as Alexander (1984) showed that it can actually range between 5 and 70%. This rate of < 25% is if there is human intervention but rates of 30-50% can occur in unsupervised flocks (Mellor & Stafford, 2004, Stafford & Gregory, 2008). Increases in herd and flock size and in productivity by individual animals have been driven by economic factors (Stafford & Gregory, 2008). The way to increase the flock size and increase productivity is most easily achieved by increasing the lambing rate of the ewe, which is favoured in intensive systems. However, this increase in litter size has also led to an increase in lamb mortality (Kouimtzis, 1985; Knight *et al.*, 1988; Lindsay *et al.,* 1988). This is noticeable as research showed that triplets had the lowest rate of survival when compared to smaller litter sizes (Cloete, 1992; Everett-Hincks *et al.,* 2005; Everett-Hincks & Dodds, 2008; Riggio *et al.*, 2008). This increase in litter size also influences essential lambing behaviours in the post-natal period (Owens *et al.*, 1985; O'Connor *et al.,* 1992; Dwyer, 2003; Dwyer *et al.,* 2005) that are crucial for survival. Triplet lambs were noted to be slower to progress to suckling than either twins or singles (Dwyer & Lawrence, 2005; Dwyer *et al.,* 2005).

Causes of neonatal mortality can include a number of factors such as birth trauma, failure of the neonate to adapt after birth, diseases and predation (Dwyer, 2008; Dwyer *et al.*, 2015), as well as the starvation-mismothering-exposure complex (SME-complex) (Haughey, 1991; Nowak & Poindron, 2006). Starvation is caused by an insufficient intake of colostrum or milk (Haughey, 1991; Mellor & Stafford, 2004), which in turn is caused by mismothering. A crucial aspect of adequate mothering is the formation of a mother-young bond (Nowak *et al.*, 2000; Napolitano *et al.*, 2008) and the ewe being able to recognise her lamb (Keller *et al.*, 2003; Sèbe *et al.,* 2007). If such a strong bond is lacking, separation is likely to occur. Lamb mortality due to dystocia was found to be just as prevalent in single lambs with heavy birth weights as it is in twin lambs that have above average birth weights, while lighter lambs are more likely to succumb with aetiology typical of the SME-complex. Together, these factors combine as the major causes of perinatal lamb mortality (Haughey, 1991; Morris *et al.,* 2000).

A potential way of improving lamb survival in a sustainable manner would be by improving both lambing and rearing practices (Sawalha *et al.,* 2007). The techniques that assess the welfare of the animal has almost exclusively been developed for animals that are reared and kept under intensive conditions, whereas animals kept under extensive condition have largely been ignored (Turner & Dwyer, 2007). The reasoning behind this is that intensive systems are generally aimed at keeping a controlled environment

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and to maximise the productivity of animals. Assessing welfare in an extensive environment poses the challenge that the environment is unpredictable and variable, which is not seen in intensive systems (Dwyer, 2009). Furthermore, a small number of breeds and crosses that have been developed for a high productivity under those controlled conditions seen in intensive systems. However, this is not the case under extensive farming conditions, where breeds are used that are adapted to that specific environment, which in turn must make the assessment of welfare highly flexible (Turner & Dwyer, 2008). The assessment of welfare relies on the expression of certain behaviours that are caused by distress, such as vocalisations. The assessment of stress or fear can be measured by the behavioural reactivity of the animals to the presence of a human (Dodd *et al.*, 2014; Brown *et al.,* 2016) or in contrived situations where animals are unfamiliar with the situation (Boissy *et al.*, 2005). This reaction is deemed to be the temperament of the animal (Réale *et al.*, 2000). The temperament of an animal can in turn have an effect on the expression of certain crucial behaviours that influence the survival of the animal as well as its young. Nervous ewes expressed fewer positive perinatal behaviour repertoires than calm ewes. Calm ewes had a longer duration of grooming their neonatal lambs, made fewer vocalisations and circled less 1-2 h after parturition (Bickell *et al.,* 2011). Although the pasture and indoor behaviour of neonatal ewes and lambs has been studied quite extensively (Gonyou & Stookey; 1985; Alexander, 1988), few studies reported genetic correlations with a cardinal welfare and production trait, like lamb survival. Similarly, few estimates link behavioural and welfare attributes to an important composite reproduction trait like number of lambs weaned per ewe mated (Brien *et al*., 2014).

The temperament or behavioural activity of the animal can be assessed through either non-restrained or restrained tests. Of particular interest in this study is the arena test (Fell & Shutt, 1989; Murphy *et al.*, 1994; Kilgour & Szantar-Coddington, 1995; Kilgour, 1998; Beausoleil *et al.,* 2008; Wolf *et al.*, 2008; Bickell *et al.,* 2009, Barnard *et al.,* 2015) and the animals' reaction to social isolation. Events that the animal deems aversive can alter that particular animal's ability to cope with subsequent stressful conditions, which is particularly noticeable if these events occur during early life stages (Napolitano *et al.*, 2008).

Against this background, the research aims of this study were to provide proof of a genetic basis for behaviour traits with the potential to improve the welfare of the local ovine genetic resource, which in turn is advocated to improve not only lamb survival but also the reproduction of the ewe. Reducing the fear in flocks may additionally improve the quality of handler-animal interactions.

An important output of the study would be to recommend actions that may benefit the welfare of animals in the wider sheep industry. To achieve this quest, the thesis has been structured to research ovine behaviour in relation to selective breeding for reproduction. Along with this the objective is to assess the relationship arena behaviour has with production traits, the relationship peri-parturient ewe and lamb behaviour has with lamb survival and the whether it is possible to improve behaviour through selection and breeding. The project team envisages that the results so obtained may be refined to improve animal welfare in tandem with product output in local sheep flocks, thereby promoting ethical production practices of free-ranging sheep in South Africa. The project team hopes that this study will contribute to a better

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understanding of the complex interactions between ethology, welfare and husbandry with reference to sheep and that it will contribute to ethical and sustainable production practices in the wider industry.

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Chapter 2
Literature Review

2.1 Introduction

To fulfil the aim set out in the previous chapter, data were sourced from different origins. Firstly, data collected on ewe and lamb behaviour in a Merino resource flock that were divergently selected for number of lambs weaned per mating (NLW) from 1993 to 2002 were considered. Data for ethological studies are known to be difficult and time-consuming to collect. Behavioural databases exceeding 1000 records that would allow genetic evaluation are thus not common. Provisional reports involving a subset of the data used here were published in proceedings of a limited scope (see Cloete *et al.,* 2003b; 2006) that were constrained by word limits imposed by the scientific committees of the meetings where it was presented. The present study therefore extends the latter reports to mature full-scale papers by the addition of data on lamb survival and number of lambs weaned per ewe mated (NLW). These records were used to add to the paucity of results involving genetic correlations of behavioural attributes with production traits. Also, because the present study is not constrained by word limits, it was possible to add supporting evidence in the form of figures and tables that allowed a much more comprehensive and informative discussion of the results that were obtained. With the recent advent of welfare as an important study field it was furthermore feasible to add an exclusive welfare element to the study.

Secondly, data on behaviour, production and reproduction stemming from an embryo transfer project were included to complete the study. As the animals in this part of the study were reared by randomly assigned surrogate dams, it was possible to assess production and behavioural responses in the divergently selected lines uncomplicated by lineage of the dam. Finally, data that were recorded over a 15-year period on the arena behaviour of young sheep were analysed and related to the divergent selection in the resource flock. These records contributed to the understanding of the reaction of animals to humans in a contrived situation. Cloete *et al.* (2010) reported provisional results in this respect at the 9th World Congress on Genetics Applied to Livestock Production during 2010 in Leipzig, Germany. The data that accrued since allowed the estimation of more accurate genetic parameters, as well as a much more comprehensive assessment of welfare aspects of the results that were reported.

The ewe's quality of maternal care given to her lamb(s) affects the survival of the lamb (Bickell *et al.*, 2009), where the formation of a close and exclusive bond between the ewe and lamb is crucial (Nowak *et al.*, 2000). Ewes are attracted to any newborn lamb in the periparturient phase, but within a few hours after birth maternal care is restricted to their own lambs and rejection of alien young is highly likely (Keller *et al.*, 2003). Two of several significant causes of deaths in lambs has been identified as abnormal ewe behaviour (Alexander, 1960; Arnold & Morgan, 1975) and lamb birth weight that could be either too low (increased risk of starvation and exposure) or too high (increased risk of dystocia; Gama *et al.,* 1991). By improving lambing and rearing management practices the management and environmental conditions at lambing can be improved, which in turn has an important effect on lamb mortality (Sawalha *et al.,* 2007). Perinatal lamb mortality varies greatly between and within breeds, flocks, seasons and management systems

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(Haughey, 1991). The ability of the ewe to give birth, with no complications, to a live lamb and to provide adequate mothering, colostrum and milk to her progeny is crucial to good lamb survival. This is particularly true as the lamb must adapt outside of the uterine environment, including standing, suckling and showing locomotor activity reasonably fast after birth (Brien *et al.*, 2014). Arnold & Morgan (1975) suggested that the lamb deaths caused by starvation may likely be the result of the ewe having no milk, or the lamb having been deserted by the ewe. Both environmental and lamb factors influence lamb survival (Brien *et al.*, 2014). The maternal behaviour of the ewe has also been identified as a contributing factor towards the survival of the lamb, where these behaviours include isolation before parturition, an uncomplicated birth process, insistent grooming of the lamb, affirming vocalisations and the assistance of the lamb to find the udder (Brown *et al.*, 2016). Selecting for multiple rearing ability has resulted in improved maternal and lamb behaviour that favours lamb survival (Cloete *et al.,* 2005a).

The temperament of the animal determines their behavioural responses to fearful- (averse response to human contact or handling) or to novel situations. Appropriate emotional reactivity and selection for reducing fear responsiveness and/or an increased flocking behaviour is important for ovine welfare and animal production as well as the improvement of it (Boissy *et al.*, 2005). Sheep show signs of significant behavioural and physiological anxiousness when isolated from their flock mates even for a short period of time, as the isolation elicits fear and can cause stress (Boissy *et al.*, 2005; Dodd *et al.*, 2012).

The survival of lambs has also been affected by selection for temperament in the ewe as temperament influences the expression of the maternal behaviour expressed by the ewe (Bickell *et al.,* 2011). Dwyer & Lawrence (2000a) concluded that the genotype of the lamb influenced the early behavioural development during the immediate postnatal period. However, after this period, the behaviour of the ewe had a significant effect on the frequency of the expression of behaviours by the lamb. The opposite effect was not seen, as the genotype of the lamb had no effect on the behaviour of the ewe. Lamb survival up until weaning was lowest for triplets, ram lambs and lambs born to 2-year-old dams (Everett-Hincks & Dodds, 2008). Ewe maternal score is positively related to average lamb weight gain to marking. This result shows that ewe temperament affects survival as well as production, as there is also a reduction in the number of lambs dying per litter at the recording of maternal score. Following on from this introduction, the reader is supplied with several chains of thought that may contribute to the development of a better understanding of the research aims proposed in Chapter 1.

2.2 Maternal Behaviour

The maternal behaviour of the ewe is influenced by several components to allow for the proper bonding between the ewe and her lamb(s) which include nursing-, responsive-, attentive-, and protective behaviour (Grandinson, 2005). The recognition of the lamb by the ewe is mediated by olfactory recognition including visual and auditory signals, which has to occur quickly after birth (Keller *et al.*, 2003). Auditory signals in sheep are mainly seen during ewe-lamb interactions, including low-pitched bleats (made by the ewe when the newborn lamb is close) and high-pitched bleats (contact or distress, when ewe and lamb

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are separated or isolated from each other or the flock) (Nowak *et al*., 2008). Visual signals allow the ewe to recognise her lamb(s) at a distance, in contrast to the ewe only accepting suckling attempts from a lamb exclusively bonded with, as mediated by olfactory recognition (Keller *et al.*, 2003). When parturition occurs, the ewe will tend to isolate herself from the flock, reduce her locomotor activity and select a birth site where she will remain for some time after lambing. During this time the ewes will become attracted to the newborn lamb and establish a strong and selective bond (Nowak, 1996).

2.2.1 Behaviour before parturition

Normally ewes will not display any maternal behaviour until a point close to parturition. However, in some cases, there may be bleating or restlessness prior to the first definite signs of impending birth (Alexander, 1988). At this stage, ewes may show interest in amniotic fluids of other ewes and/or lambs (Nowak *et al.*, 2008). In some instances, ewes may display interest in foreign lambs and may clean/groom them. In most cases, this interest will suddenly develop just before parturition, but in rare instances such behaviour may be observed as early as 2 weeks before lambing. The expression of this behaviour may result in interference with other ewes or lambs which could lead to confusion over which ewe the lamb belongs to (Alexander, 1988). Ewes still to lamb may adopt foreign lambs which may, in certain instances, compromise the maternal care of their own offspring. Rejection of either the adopted lamb or the ewe's own lamb after parturition could easily happen under these conditions (Alexander, 1960). Maternal interest before lambing was almost exclusively observed within 8 h of lambing and mostly within 2 h. These interests ranged from grooming, brief inspection, suckling and in some cases attempted adoption of another lamb (Arnold & Morgan, 1975).

The isolation and shelter seeking behaviour, as well as the selection of the birth site and the time spent on the birth site, will differ between breeds and individuals. The ewe separates from the flock as parturition approaches (Alexander, 1988). This behaviour may also be influenced by the parity and experience of the ewe (Brien *et al.*, 2014), as well as, in some cases, by litter size. However, it is not clear if the ewe actively chooses to separate from the flock or if she gets left behind (Nowak *et al.*, 2008). Alexander *et al.* (1990) also reported that multiparous ewes will seek isolation prior to birth more readily than primiparous ewes. Experienced ewes will also remain on or near the birth site for longer than inexperienced ewes (Alexander *et al.,* 1984). In a study done on Merino ewes, Stevens *et al.* (1982) reported that ewes that had single or twin lambs remained at the birth site for several hours, where grooming and suckling behaviour was observed. Most of the ewes that were observed remained at the birth site for 2-3 h after lambing. However, in a study done by Alexander *et al.* (1983), there were no clear differences between the period remained at the birth site between ewes with singles and ewes with twins, but the mean time of 2 h spent at the birth site corresponded with the results of Stevens *et al.* (1982). Twin-bearing ewes chose a sheltered lambing site more readily when compared to ewes with single lambs (Knight *et al.*, 1988).

In a study done by Gonyou & Stookey (1985) on ewes in an intensive system, it was found that 41% of ewes chose to lamb in cubicles provided, and those that had been exposed to the cubicles in previous

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years tended to uses them more readily. The authors argued that the ewes chose the cubicles because of the reduced number of ewes as well as the protection that it provides when compared to the larger pen the cubicles were situated in. The ewes that lambed in the cubicles had less interference while being with their lambs and remained longer at the birth site. Ewes that eventually chose to lamb in cubicles spent more time in the cubicle, stopped at fewer potential lambing sites, travelled less and spent less time moving than ewes giving birth in the pen outside the cubicles.

Under extensive conditions, the selection of the birth site is important as it contributes to the survival of the lamb, particularly under adverse conditions, and determines the micro-environment the lamb will be born in (Watson *et al.,* 1968; Alexander, 1988; Dwyer & Lawrence, 2005; Dwyer *et al.*, 2015). Lynch & Alexander (1976) reported that shorn and unshorn ewes used the shelter provided for different reasons and showed different patterns of shelter use during both the day and night. Similarly, Stevens *et al.* (1981) reported that if shorn ewes were provided with shelter under paddock conditions a significantly higher proportion of shorn ewes chose to lamb away from the group. Alexander *et al.* (1979) reported that unshorn ewes tended to lamb in larger paddocks away from other sheep. This behaviour was also seen in smaller paddocks, whereas the shorn ewes lambed in proximity to other sheep in the larger paddocks, especially where they were most concentrated. A larger concentration of birth sites was located near to the paddock fences rather than in the middle, indicating that preference of ewes to lamb in isolation away from other sheep may be restricted by the fences.

Selection of birth sites and time spent on the site have been shown to differ between breeds as well. Alexander *et al.* (1990) reported that Merino ewes were randomly distributed when the pasture was level, but when the pasture was sloped, the ewes preferred a birth site that was elevated. Upland as well as lowland ewes were shown to prefer lambing sites at the edge of the pastures. Knight *et al.* (1988) reported line differences in selection of lambing sites, as more Marshall Romney ewes chose sheltered lambing sites compared to control Romney ewes. The Marshall Romney is a line that has been selected for an improved maternal rearing ability. Stevens *et al.* (1981) showed that relatively few fine-wool Merino ewes will choose to lamb in isolation. Merino ewes spent less time on or near the birth site (Alexander *et al.,* 1983) and SA Mutton Merinos was seen to remain at the birth site for a shorter period of time than Dormers of the same flock (Cloete, 1992). Merino ewes that lambed in sheltered areas generally remained at the birth site longer when compared to the ewes that lambed in the open (Alexander *et al.,* 1983).

Birth site selection has been confirmed as being an important factor in preventing the separation of ewes and their lambs (Dwyer *et al.*, 2015) and will therefore contribute to the survival of the lamb by enhancing the attachment and recognition between ewes and their lambs. Putu *et al.* (1986) found that if the ewes were moved from their birth sites 30 min after the second lamb was born, there was an increase in the number of ewes that deserted their lambs, particularly second-born multiples. Ewes which stayed at the birth site for 6 h had less desertions and were more likely to reunite with their lambs if there were separations. Similarly, Murphy *et al.* (1994) showed that the survival of multiple-born lambs was better when ewes and lambs remained in close contact for the first 6 h after birth. Ewes would adjust their pace in order for the lamb to be able to keep up and in some cases would not move far unless both twin lambs

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were present, but instances where ewes waited for newborn lambs were unusual (Stevens *et al.,* 1982). Cloete (1992) found that separations were more likely to occur if the ewe remained on the birth site for shorter than 2 h when compared to the ewes that remained on the birth site for longer than 2 h.

The sheltering behaviour of the ewe may be varied, and is normally prompted by the weather (too cold or too hot). Pollard *et al.* (1999) found that the shelter-seeking behaviour of the ewe was significantly associated with weather factors such as temperature, rain and wind. The occurrence of shelter-seeking behaviour increased after lambing and decreased slowly as the lamb aged.

2.2.2 Behaviour at and just after parturition and the factors that affect it

2.2.2.1 Recognition of lamb

Parturient ewes will show an attraction to the olfactory and other sensory cues provided by the neonatal lamb, which includes licking and grooming of the lamb, frequent low-pitched bleats and standing still to facilitate the lamb finding the udder. Keller *et al.* (2003) concluded in their study that both selectivity at sucking and recognition of the lamb at a distance is established very quickly after parturition. They also found that the experience of the ewe influenced olfactory and visual/auditory recognition differently. Olfactory recognition was effective 30 min after parturition, involved a rapid learning process, and was not influenced by the maternal experience of the ewe. Visual/auditory recognition was established as early as 6 h postpartum in multiparous ewes and develops slower in primiparous ewes. The authors thus concluded that visual/auditory recognition is enhanced by previous maternal experience of the ewe. The recognition of lambs by their dams after lambing was important to prevent lambs from separating from their dams and to avoid the adoption and care of alien lambs (Sèbe *et al .,* 2007).

2.2.2.2 Desertions of lambs

Evidence suggest that ewe behaviour was affected by the length of parturition as ewes with longer parturition times and stressful births tended to take longer to stand after lambing than ewes with a shorter parturition time (Shelley, 1970). Similarly, long and complicated births led to failure to show adequate maternal care and more incidences of abandoned lambs, compared to short and unassisted births (Darwish & Ashmawy, 2011). Owens *et al.* (1985) found that 2-year-old ewes took longer to stand after the birth of the first-born lamb of all litter sizes than older ewes. Desertion was also affected by the length of parturition as ewes having longer parturition times tended to desert their lambs more often than ewes with shorter parturition times (Shelley, 1970). Alexander (1960) observed that ewes with longer parturition times showed less concern when the lamb is removed. Desertions also occurred more frequently in ewes with longer parturitions (Shelley, 1970). Ewes with stillborn lambs may display an increased interest in other lambs and may adopt them.

Desertion of lambs was mostly seen in 2-year-old ewes and no older ewe in a study by Alexander (1960) deserted her lamb. In cases of adoptions of lambs, it was observed to be more frequent in ewes that had still-born lambs while deserted lambs that were a few hours old were also adopted (Alexander, 1960).

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2.2.2.3 Assistance required at birth

Breed significantly affected the need of ewes to require assistance during delivery or not (Dwyer & Bünger, 2012). Assistance at birth was more prevalent in SA Mutton Merino ewes than in Dormers while SA Mutton Merino ewes had longer parturitions (Cloete, 1992). Cloete & Scholtz (1998) accordingly reported line differences for assistance at birth for maiden Merino ewes divergently selected for number of lambs weaned per ewe mated (NLW). Breeds selected for terminal sire traits (Texel and Suffolk) showed an impaired ability to lamb without assistance and had lower lamb vigour than the other breeds used in the study (Scottish Blackface and Mule x Texel; Dwyer & Bünger, 2012). The authors concluded that it is possible that assisting ewes during birth may have reduced the intensity of natural selection for birthing ease and large pelvic dimensions in ewes. This sentiment was also shared by Haughey (1991).

2.2.2.4 Vocalisation behaviour

Vocalisations by the ewe and the lamb occurred after parturition, peaked up until 3 h after parturition and declined rapidly by 12 h after parturition (Sèbe *et al.,* 2007). Low-pitched bleats increased over the first 30 min following birth and gradually declined thereafter for both single- and twin-bearing ewes (Dwyer *et al.,* 1998). A significant difference existed between the proportions of the types of vocalisations. Low-pitched bleats were only heard after parturition and were generally only heard in the presence of the lamb (Dwyer *et al.,* 1998). Intermediate bleats were less frequent than both high- and low-pitched bleats, and low-pitched bleats were more frequent than high-pitched bleats (Sèbe *et al.*, 2007). Ewes could recognise and had a preference for their own lamb's bleat within 24 hr from parturition whereas lambs only started to show preference for specific dams at 48 h (Sèbe *et al.*, 2007).

Breed differences, as well as ewe parity, have been found in a study by Dwyer *et al.* (1998), where primiparous Scottish Blackface ewes had a higher bleating rate than primiparous Suffolk ewes during the first 2 h after birth. The primiparous Scottish Blackface ewes also uttered more low-pitched bleats than the multiparous ewes. Fewer high-pitched bleats were observed in single-bearing Scottish Blackface ewes whereas an increase in high-bleats were observed in their Suffolk contemporaries when ewe experienced was considered. Suffolk ewes bearing twins tended to bleat more than Blackface ewes. In a study by Keller *et al.* (2003), primiparous ewes emitted significantly more bleats in the presence of their lamb(s) and suckled their lamb(s) for longer than both biparous and multiparous ewes when selectivity at suckling and recognition tests were conducted. Everett-Hincks *et al.* (2005) reported that the ewes with triplets uttered significantly more high-pitched bleats than the ewes with twins.

2.2.2.5 Grooming behaviour

Grooming and cleaning of lambs commenced immediately after lambing (Arnold & Morgan, 1975) and continued while the lamb attempted to stand and reach the udder (Alexander, 1960). Any delay in grooming behaviour was associated with a longer labour (Arnold & Morgan, 1975; Darwish & Ashmawy, 2011) as the interval between birth and the commencement of grooming may lengthen if the ewe were

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lying for some time after birth, likely caused by exhaustion. Dwyer & Lawrence (1998) reported that there was intensive grooming attention after birth, but that it gradually declined over time. However, a significant portion of ewes' time was spent on grooming the lamb up to 2 h after birth.

The grooming behaviour of the ewe differed between breeds. In breeds such as the Romanov and Préalpes du Sud, the ewes spent more time licking their lambs than both the Ille-de-France and the Lacaune (Poindron *et al.,* 1984; Le Neindre *et al.*, 1998). The same trend was evident in British breeds, where Scottish Blackface ewes showed increased grooming behaviour relative to the Suffolk breed (Dwyer & Lawrence, 1998, 1999a, 2000a; Dwyer *et al.*, 1998). Dwyer & Lawrence (1998) reported that both Scottish Blackface and Suffolk ewes exhibited a reduction in grooming attention towards a first-born lamb at the birth of a subsequent litter mate. If the second twin was born after 45 min, however, it received less grooming attention than those born first. This was, however, not seen when twins were born within 10 min of each other as they received equal amounts of grooming, but still less than would be expected for a single lamb. Scottish Blackface ewes showed a slight increase in their grooming attention to twin lambs in the first 30 min after birth, but this was not seen in Suffolk ewes. Scottish Blackface ewes also commenced with grooming their lambs quicker than Suffolk ewes (Pickup & Dwyer, 2011). Suffolk ewes started to begin grooming more slowly and did it in shorter bouts (Dwyer, 2008). In a study done on Merino ewes (Alexander, 1960), grooming behaviour occurred immediately after birth and most ewes expressed this behaviour within 2 min of birth and continued while the lamb attempted to stand and locate the udder. The quick commencement of grooming behaviour in Merino ewes were also seen in a study by Bickell *et al.* (2009) where all but one ewe commenced grooming within 10 min of birth, as well as in Booroola Merina ewes which commenced grooming within 4 min of birth (Owens *et al.,* 1985).

Sex of the lamb had no effect on the amount of time ewes spent on grooming the lamb (O'Connor *et al.,* 1992), and singles and first-born twins received the same amount of grooming time after birth. However, a difference was seen with second-born twins, who received significantly less grooming attention during the first 10 min after birth. First-born twins also received much less grooming attention at 30 min than singles when the second twin is born.

No differences were found between multiparous and primiparous ewes in their time spent grooming singles. However, differences were seen with the birth of the second lamb. Multiparous ewes groomed newborn second twins more than primiparous ewes, while primiparous ewes showed more grooming attention to the first-born twin at this time than multiparous ewes (O'Connor *et al.,* 1992). Primiparous ewes were slower to begin grooming and licking their lambs in studies by Dwyer & Lawrence (1998, 2000b).

2.2.2.6 Behaviour during suckling attempts

Butting and failure to stand still (to facilitate suckling) was observed in 2-year-old ewes. Butting occurred when the lamb moved and failure to stand still was defined as when the ewe turned to face the lamb as it tried to reach the udder. The butting ceased after an hour or two, whilst failure to stand persisted until 6-12 h (Alexander, 1960). In ewes that had short or unassisted births, there was a higher incidence

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of acceptance of suckling by lambs when compared to ewes that had prolonged and assisted births (Darwish & Ashmawy, 2011).

Breed differences can occur when these behaviours are observed. The most notable differences have been found between Scottish Blackface ewes and Suffolk ewes. When negative behaviour was observed, Suffolk ewes, both primiparous and multiparous, tended to be more likely to reject at least one lamb, stood still and allowed the lamb to suck for proportionately less sucking attempts, were significantly more likely to butt the lamb, back away, and withdraw from their lambs than Scottish Blackface ewes (Dwyer & Lawrence, 1998, 2000a, 2000b; Dwyer, 2008). Improved maternal cooperation was showed by Scottish Blackface ewes with the early attempts to locate the udder and suckle (Pickup & Dwyer, 2011), but those with single lambs moved more in response to sucking attempts (Dwyer & Lawrence, 1998, 1999a; Dwyer, 2008). The most prevalent ewe behaviour to sucking attempts was circling (Dwyer & Lawrence, 1998, 1999a). Scottish Blackface ewes primarily circled around the lamb (O'Connor *et al.,* 1992; Dwyer & Lawrence, 1999a), a behavioural attribute occurring more in primiparous ewes than in multiparous ewes. Backing away from lamb(s) was not seen in multiparous ewes (O'Connor *et al.,* 1992).

In the incidence of twins, after the birth of the first-born lamb, ewes stood for a relatively smaller proportion of suckling attempts, whereas a breed difference was seen after the birth of the second-born twin, where Scottish Blackface ewes stood more readily for suckling attempts than Suffolk ewes (Dwyer & Lawrence, 1998). Lambs that had a Scottish Blackface dam had proportionately more successful suckling attempts than lambs with a Suffolk dam (Pickup & Dwyer, 2011).

2.2.2.7 Incidence of separations

Merino ewes had significantly more permanent separations from one of their twin lambs than other breeds, where most of the separations occurred within 24 h of lambing. In some cases permanent separation was caused by the lamb itself, by lambing difficulties, or by other sheep interfering, whereas in the remaining cases no obvious factor could be associated with the separation (Alexander *et al.,* 1983). In Romney ewe, separation of lambs from their dams was only observed in ewes lambing for the first time (Alexander *et al.,* 1984). In Merino ewes selected for temperament (calm or nervous) no difference was observed in their ability to recognise their own lambs in separation or choice tests. During the initial stages of these tests the ewes with nervous temperaments were more prone to react positively (Bickell *et al.,* 2009). In a study by Alexander *et al.* (1983) the authors reported that fine-wool Merino ewes were separated much more readily from their lambs than other breeds used when maintained under the same pasture conditions. In the same study the authors concluded that several factors contributed to whether the ewe will separate from the lamb including birth problems as well as interference from other ewes and lambs. Ewes with twin lambs, especially Merino ewes, were less likely to attend to both lambs and to ensure that both followed when they moved.

Among 2-year-old ewes that bore twins, separation from at least one twin was also highest in ewes lambing for the first time. Separation from a twin lamb was lowest in the older ewes and in ewes that had previously produced twin lambs (Alexander *et al.,* 1984). Alexander *et al.* (1984) suggested that the ewes'

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ability to care for twin lambs improved rather with experience than with age, as inexperienced ewes were 2-3 times more likely to separate from at least one of her twin lambs. Primiparous ewes were more likely to desert their lamb's (Dwyer & Lawrence, 1998). In a study where lambs and ewes were separated at birth and reunited after a set interval to assess the acceptance of the lambs by the ewes (Smith *et al.,* 1966), it was reported that the older ewes who have had several previous lambing experiences accepted their lambs more readily than younger ewes.

Shillito & Alexander (1975) compared the ability of both ewes and lambs to recognise their own as well as alien ewes or lambs. For ewes the appearance of the lamb had a significant effect indicating that if an alien lamb was of similar appearance/colour to the ewe's own lamb the scores were higher. If the lamb of the ewe had a different appearance/colour to the ewe, the ewe would show little to no interest in the alien lamb. The same effect was seen when comparing the approach or interest of the lamb to an ewe. The age of the lambs also had an effect on their behaviour as very young lambs stood still as ewes approached. In contrast, older lambs would move towards the ewe and would often run to the ewe. Poindron *et al.* (1979) showed that the spontaneous acceptance of the lamb by the ewe and the proportion of cases it was found depended on the length of time that the ewe and the lamb were separated after birth.

2.2.3 Maternal Behaviour Score

The Maternal Behaviour Score (MBS) is normally measured within 24 h after birth when lambs are tagged. It is measured by observing the distance that the ewe keeps from her lamb, the closer the ewe is to her lamb and the handler the better the MBS score is. O'Connor *et al.* (1985) measured MBS on a 5-point scale, namely: 1) ewe flees as shepherd approaches and does not return or show interest in her lamb(s); 2) ewe retreats further than 10 m but returns to her lamb(s) as the shepherd leaves; 3) ewe retreats to a distance where tag identification is difficult (5 – 10 m); 4) ewe retreats but stays within 5 m of lamb(s) and shepherd; 5) ewe stays close to her lamb(s) as the shepherd is handling them. It is a relatively quick and easy to use and give a score and does not add a significant amount of time it takes to collect lambing and pedigree information, therefore making it relatively attractive to use (Brien *et al.*, 2014).

O'Connor *et al.* (1985) found that as MBS increased, the survival of the lambs increased from birth to weaning. Progeny of ewes with an MBS of 1 had significantly lower survival rates from birth to weaning than those of ewes of higher MBS. The study also showed that bigger litters tended to have lower survival rates from birth to weaning.

MBS was significantly affected by ewe age and genotype as well litter size, as MBS increased with each year increase in ewe age and for each unit increase in litter size (O'Connor *et al.*, 1985). This effect of litter size on MBS was also observed in a study by Everett-Hincks *et al.* (2005), where the authors reported that the score increased in ewes with triplets when compared to the ewes with singles and twins. Lambe *et al.* (2001) found that single-bearing ewes had lower MBS than ewes bearing multiples. Brown *et al.* (2016) found that MBS improved significantly as the age of the ewe increased and the authors found that ewes that were born as singles had higher MBS scores.

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When lambs were tagged in a study by Alexander *et al.* (1983) they observed that half of the ewes of every breed used in the study (Merino, Dorset, Border Leicester X Merino) moved 20-50 m away from the handler but did return to their lamb(s) once the handler left. It was also seen that a higher proportion of Dorset ewes remained with their lamb when a handler was present than both Merino and crossbred ewes. Romney ewes stayed within 5 m of the handler at lamb tagging and, in some cases, remained with the lamb.

Bleating frequency influenced MBS as the ewes with a lower MBS made more high-pitched bleats (Everett-Hincks *et al.* 2005). Lambe *et al.* (2001) showed that the MBS of the ewe had no significant effect on the average weight gain of the lambs reared to marking as well as to weaning, and that there were no significant differences between the average weight gain of lambs between any of the MBS categories.

2.3 Lamb behaviour

Lambs may attempt to stand within a few minutes of birth and will normally do so within 30 min of birth, they will succeed in suckling within 30-45 min after standing and by approximately 90 min after birth (Arnold & Morgan, 1975). Lambs that had short and unassisted births were significantly more active in the first 2 h following parturition, as they stood and suckled quicker when compared to lambs with long or assisted births (Darwish & Ashmawy, 2011). Assistance during birth also affected the vocalisation behaviour, as lambs with assisted births had a higher bleat frequency than lambs that did not require assistance (Darwish & Ashmawy, 2011).

The latency to get upright to their knees and start bleating did not differ between two temperament lines for lambs born in a flock selected divergently for calm and nervous temperaments (Bickell *et al.,* 2009). There was however a difference in the lamb's latency to suckle, where lambs in the nervous line were quicker to start suckling that calm lambs. However, there was no line difference in the amount of time spent suckling. Nervous line lambs were in general also quicker to stand and attempt to stand compared to calm lambs (Bickell *et al.,* 2009).

The major influence on the activity of the lamb and its behaviour is the breed of the lamb. As is seen in the ewes, Suffolk and Scottish Blackface lambs also exhibited differences in their behaviour. Scottish Blackface lambs were more active within the first 30 min after birth than Suffolk lambs (Dwyer & Lawrence, 2000a). Suffolk lambs took longer to stand and suckle after birth than Scottish Blackface lambs (Dwyer & Lawrence, 1998, 1999a, 2000a; Dwyer *et al.,* 1998). They also made significantly fewer attempts to suckle than Scottish Blackface lambs (Dwyer & Lawrence, 1998, 1999a, 2000a).

Male lambs were slower to stand, reach the udder, attempt to suckle, and successfully suckle than female lambs (Dwyer & Lawrence, 1998; Dwyer, 2003, Dwyer *et al.,* 2005). Lamb sex had an effect in Suffolk lambs (male lambs being slower to perform normal lamb behaviours) but not in Scottish Blackface lambs (Dwyer, 2003; Dwyer *et al.,* 2005).

Lower levels of activity were particularly prevalent in lambs that required assistance during birth as they were less active than unassisted lambs during this first 30 min after birth (Dwyer & Lawrence, 1999a).

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Birth weight in Suffolk lambs was associated with an increase in the speed at which the lamb stood and suckled, whereas birth weight was only significant for suckling behaviour in Scottish Blackface lambs (Dwyer, 2003) .

Twin Suffolk lambs had a higher bleat rate than twin Scottish Blackface lambs. Birth order also affected the bleat rate as first-born lambs of twins and single-born lambs had similar bleat rates while second-born twins increased the mean bleat rate (Dwyer *et al.,* 1998). Dwyer & Lawrence (1998) reported no significant effect of litter size on the time it took for the lamb to stand, time to reach the udder, time to attempt to suckle, and the time it took to successfully suckle. This was, however, not the case in other results found in literature (Owens *et al.*, 1985; O'Connor *et al.,* 1992; Dwyer, 2003; Dwyer *et al.,* 2005) where litter size had a significant effect on all the behaviours of lambs. Twin lambs tended to be quicker in their initial behaviours after birth than both single and triplet lambs (Dwyer *et al.,* 2005). However, O'Connor *et al.* (1992) found that singles were more active and made significantly more suckling attempts at 90 min than twin lambs. The time it took to stand, to find the udder and attempt to suckle, was affected by litter size but also by birth order. Single lambs and first-born twin lambs were quicker than first-born lambs of both triplet and quadruplet litters and second-born lambs of twins were quicker to suckle than the second-born lamb of triplet and quadruplet litters (Owens *et al.*, 1985).

The dam, specifically the breed, had a significant effect on the time taken by Suffolk lambs to stand (Dwyer & Lawrence, 1999a, 2000a), as Suffolk lambs born to Scottish Blackface ewes were significantly slower to stand compared to if they were born to Suffolk ewes. Suffolk ewes received a significantly greater number of sucking attempts than Blackface ewes (Dwyer & Lawrence, 2000a). Grooming behaviour of ewes was also associated with a delay in the lamb being able to stand (Dwyer & Lawrence, 1999a). Arnold & Morgan (1975) suggested that the behaviour of the ewe may influence the behaviour of the lamb by either restricting or denying access to the udder or by prolonging the time it takes for the lamb to suckle. The behaviour of the ewe had a negligible effect on the initial udder seeking behaviour, however, there was a significant effect of the ewe breed on the frequency of the sucking interactions.

An increase in experience or parity of the ewe is associated with a reduction in the time it takes the lamb to perform righting, standing, udder-seeking, and sucking behaviours (Dwyer *et al.*, 2005). Lambs born to first parity ewes had a higher sucking frequency than lambs born to multiparous ewes, but this frequency declined as the number of pregnancies of the ewe increased (Dwyer *et al.*, 2003). The experience of ewes could have a significant effect on the bleat rate of the lamb, as reported by Dwyer *et al.* (1998). Lambs born to both primiparous Suffolk and Blackface ewes had a higher bleat rate than lambs born to multiparous ewes. First-born lambs of all litter sizes of 2-year-old ewes took longer to stand than those cared for by older ewes (Owens *et al.,* 1985).

2.4 Lamb survival

The bond between the ewe and the lamb is essential for its survival as it facilitates the early suckling behaviours and the subsequent intake of colostrum (Nowak *et al.*, 2000), as does the selection of the birth

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site. Lamb mortality differed between breeds and crosses, as well as between strains between breeds, when compared under similar environmental conditions (Alexander, 1984).

2.4.1 Factors affecting lamb survival

Several factors have been indicated in whether the lamb survives or not. Both dam and lamb behaviour play a pivotal role in the survival of the lamb, but both management and environmental conditions have been shown to affect lamb mortality. A potential way of improving lamb survival could be by improving both lambing and rearing practices (Sawalha *et al.,* 2007). Lamb losses normally occur in the first 3 days after birth (Cloete, 1992; Nowak *et al.*, 2000; Sawalha *et al.,* 2007; Brien *et al.*, 2010) and the causes are usually related to factors such as trauma during birth, failure of the neonate to adapt to postnatal life, infectious diseases, and predation (Dwyer, 2008; Dwyer *et al.*, 2015).

Another factor that contributes to neonatal mortality is the starvation-mismothering-exposure complex (SME complex). Mortalities that are due to this complex are caused by hypothermia accompanied by the absence of, or inadequate amounts of milk intake (Haughey, 1991), adverse weather conditions, inadequate energy reserves, problems with thermoregulation, delayed lactogenesis, insufficient colostrum yield, negative maternal and lamb behaviour, and udder defects (Nowak & Poindron, 2006). Morris *et al.* (2000) suggested that the low rates of survival due to dystocia was just as prevalent in heavy twin lambs as it is in heavy singles. Singles that had below average birth weights had similarly low rates of survival which indicated that mismothering, starvation and/or exposure were potential problems for al light lambs (Morris *et al.,* 2000).

Assistance during birth has also been contributed to a higher lamb mortality rate in the first week of life, than when no assistance is given (Darwish & Ashmawy, 2011). Differences between breeds relating to factors that affect lamb survival have also been seen to have an effect (Dwyer & Bünger, 2012).The survival of a litter tended to increase from 2 to 4 years of age and then decreased while the survival of the litter also decreased as the size of the litter increased (Everett-Hincks *et al.,* 2005). Nowak (1996) indicated that there are two approaches to control mortality of lambs due to behavioural factors: 1) improve the physical conditions at and around lambing to ensure maximal survival, even if optimal maternal care is not provided, and 2) to ensure conditions to improve the early mother-young relationship, which will ensure that the lamb has the best chance of survival even when circumstances are suboptimal.

2.4.1.1 Lamb factors including birth weight, litter size, sex and breed

The birth weight of stillborn lambs was not above average even if the birth process took longer than normal, however most lambs that died without standing had a lower birth weight than those that survived (Arnold & Morgan, 1975). Lambs that had an average birth weight had a higher rate of survival, as single lambs that had a heavier birth weight and twins that had a below average birth weight had a lower survival rate than lambs with average birth weights (Morris *et al.*, 2000). The assistance required at birth was affected by the weight of the lamb, as lighter lambs went unassisted during the birth process when compared to lambs that required assistance (Dwyer, 2003; Dwyer & Bünger, 2012) and it was seen that

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the lambs that required sucking assistance were significantly lighter than those who did not (Dwyer & Bünger, 2012).An increase in productivity is favoured in intensive systems which can be partially achieved by increasing the lambing rate per ewe. This increase in litter size leads to an increase in lamb mortality which is not entirely explained by the effects of birth weight (Kouimtzis, 1985; Knight *et al.*, 1988; Lindsay *et al.,* 1988). Twin lambs had the highest rate of survival in some studies (Lopez-Villalobos & Garrick, 1999; Sawalha *et al.,* 2007). However, Knight *et al.,* (1989) reported that single lambs had a significantly higher survival rate compared to twin lambs. Cloete (1992) found no difference between the mortality rates of single and twin lambs. Stevens *et al.* (1982) reported that the mortality of second-born twins was almost 3 times that of the first-born lamb in twin litters. Triplets had the lowest rate of survival compared to twin lambs (Cloete, 1992; Everett-Hincks *et al.,* 2005; Everett-Hincks & Dodds, 2008; Riggio *et al.*, 2008).

Lambs born as singles required more assistance during birth than larger litters (Dwyer & Bünger, 2012), had a lower probability to survive than lambs that were unassisted. Triplet lambs that required birthing assistance had a lower probability to survive to survive to 1 week after birth. (Cloete, 1992). Twin and single lambs required less sucking assistance than lambs born in larger litters, but the assistance required did not differ between single and twin lambs (Dwyer & Bünger, 2012; Matheson *et al.,* 2012).

Sex of the lambs have been found to affect the survival of the lamb to a certain extent, as studies have found that male lambs tended to have a higher mean mortality rate than female lambs (Hight & Jury, 1970; Knight *et al.,* 1988; Cloete, 1992; Sawalha *et al.,* 2007; Riggio *et al.,* 2008), and that male lamb survival up until weaning was lower than that of females (Lopez-Villalobos & Garrick, 1999; Everett-Hincks & Dodds, 2008). Sawalha *et al.* (2007) noted that the sex of the lamb only actually started to play a role in survival in the period after weaning when rams and ewes are managed differently. Male lambs required significantly more birthing assistance than female lambs (Dwyer & Bünger, 2012; Matheson *et al.*, 2012), but there was no significant differences in the ability of ram and ewe lambs to suck unaided (Matheson *et al.*, 2012),

Differences between breeds were found in a study by Dwyer & Bünger (2012), using Scottish Blackface (selected for hardiness), terminal sire breeds (Texel and Suffolk), and crossbred lambs (Mule X Texel). Suffolk and crossbred lambs were the heaviest at birth, followed by Texel lambs, and Scottish Blackface lambs. The single lambs and heavier lambs in the terminal sire breeds were more likely to require assistance at birth, and these breeds, including the ram lambs of these breeds, required more assistance during sucking. Only in the Suffolk breed was it observed that lambs requiring birthing assistance would also require sucking assistance. Suffolk lambs born as singletons were significantly more likely to experience difficult births than twins (Dwyer & Lawrence, 1998). For the Scottish Blackface and crossbred lambs, only lambs born in multiple litters required assistance at birth, and lambs of these breeds were more likely to suck unaided (Dwyer & Bünger, 2012). SA Mutton Merino ewes gave birth to heavier litters than Dormers, while the age of the ewe also affected birth weight (Cloete, 1992). Knight *et al.* (1988) compared Marshall Romney (MR) ewes and control Romney (CR) ewes. The MR was selected for the ability to survive, to lamb unassisted, and to rear lambs with minimal shepherding under harsh conditions

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(MacKereth, 1979). Knight *et al.* (1988) found that lambs born to MR ewes, and that were sired by MR rams, had a higher survival rate than lambs born to CR ewes and lambs sired by CR rams.

2.4.1.2 Maternal factors

Dwyer & Lawrence (2005) summarised that the maternal behaviours associated with survival of the lambs were grooming, low-pitched bleating, showing no aggression, adequate maternal care to the lamb, cooperation with attempts to suckle, recognition of the lambs and the ewe maintaining a close contact with the lamb(s).

Separation from the ewe can compromise the survival of lambs. Stevens *et al.* (1982) reported that weak lambs were left behind. The authors couldn't conclude on whether the survival of both twin lambs is dependent on the lamb's behaviour or on the following of the ewe by the lambs, or if it was because of the ewe's failure to ensure that both her lambs are following. In twin-bearing Merino ewes it was, however, seen that the major factor contributing to separation of ewes from their lamb(s) is the ewe not attending to the lambs and ensuring that both lambs were following. Even if the lambs were strong and healthy this separation was mainly maternal. An extended period of time spent at or close to the birth site facilitated dam-offspring bonding and countered such problems (Alexander *et al.,* 1983).

Lamb survival up to weaning for all lambs were significantly affected by Maternal Behaviour Score (MBS) (Everett-Hincks & Dodds, 2008). Brown *et al.* (2016) suggested that selecting for MBS could improve the survival of lambs, especially in breeds such as the Merino, where twin lamb survival is often low. When taking Maternal Behaviour Score (MBS) into consideration it was seen that ewes with an MBS of 1 had a significantly higher mortality rate of their progeny than ewes with a higher score. However, this may not be an indication that the ewe lacks the maternal ability to care for the lamb or provide less maternal care, but rather that it is not expected of the ewe to pay much attention to a dead lamb as to a lamb that is born alive. This may be the reason for the ewes being assigned an MBS of 1 (Sawalha *et al.,* 2007*)*. Triplet lambs were at a greater risk with dams that have MBS's lower than 3 as they had lower survival rates up until 3 days of age (Everett-Hincks & Dodds, 2008). Everett-Hincks *et al.* (2005) reported that litter survival increased as MBS increased, a contention that was supported by Lambe *et al.* (2001), and that litter survival as well as lamb survival improved substantially in dams that had an MBS of 3 and above when compared to dams that had an MBS of 1 or 2. As soon as MBS increases above 3, the effect it has on litter survival becomes minimal. MBS of the dam also influenced the survival of the individual lambs, as individual lamb mortality decreased as the dam's MBS increased (Everett-Hincks *et al.,* 2005). However, single-born lambs were not affected by the MBS of their dam (Everett-Hincks & Dodds, 2008). Everett-Hincks *et al.* (2005) reported that the lambs that were born to ewes with a higher MBS had quicker ewe-lamb contact times at tagging, stood and located the udder sooner, and were significantly closer to their dams 5 min after tagging than lambs born to ewes with low MBS.

Age of the ewe has also been taken into consideration for the effect it has on the survival on the lamb. Lambs born from 2-year-old ewes were less likely to survive than lambs produced by ewes that were older (Hight & Jury, 1970; Knight *et al.*, 1988; Murphy *et al.,* 1994; Lopez-Villalobos & Garrick, 1999; Riggio

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et al., 2008), but reached a maximum in 5-year-old dams (Hight & Jury, 1970) and declined rapidly for 6-year-old ewes (Knight *et al.,* 1988). Cloete (1992) reported that there were higher overall levels of lamb mortality in older ewes than in younger ewes. Lambs born to 5-year-old dams had the lowest survival up until 3 days of age compared to other ewe ages (Everett-Hincks & Dodds, 2008). Lambs born to older ewes also required less assistance at birth (Dwyer & Bünger, 2012) but did require more assistance at suckling (Matheson *et al.*, 2012). Knight *et al.* (1988) found the selection of a sheltered birth site over an exposed birth site had no effect on the survival of the lamb.

2.5 Temperament of the ewe and lamb

The reaction of an individual to a novel environment or a challenging situation is referred to as the temperament of the individual (Réale *et al.*, 2000). Temperament may also be defined as the behavioural expression of fearfulness when the ewe or lamb is being handled or has contact with humans (Brown *et al.*, 2016). Dodd *et al.* (2014) described the behavioural reactivity of an animal as the behavioural response to stress. Selecting for temperament may improve ease of handling in both more open and restricted spaces, and is believed to be linked to production traits (Dodd *et al.*, 2012)..

In sheep flocks where ewes were selected either for a calm or nervous temperament (as defined by Bickell *et al.,* 2009), it was seen that calm ewes spent more time after birth (1 h and 2h postpartum) licking their lambs, emitted less low- and high-pitched bleats and circled less compared to nervous ewes (Bickell *et al.,* 2011). Bickell *et al.* (2011) concluded that maternal behaviours in ewes selected for temperament (calm vs. nervous temperament) was not different under controlled conditions, as ewes from both lines displayed the full range of behaviours expected at parturition. The behaviour of the lamb also was not strongly affected by selection for temperament in the same study. The only difference observed was that the ‘nervous’ lambs were more active and reached the udder sooner than ‘calm’ lambs. When socially isolated, sheep tended to become more vocally active and show behavioural changes. The time spent licking the lamb, the frequency of low-pitched bleats, the probability that ewes would prevent suckling or time at the udder, as well as the proportion of ewes that backed away from their lamb(s) did not differ between calm and nervous ewes. There was an increase in behavioural withdrawal and, if the isolation is prolonged, there may be a reduced intake of water and food (Nowak *et al.*, 2008).

Sheep were also selected according to locomotor activity in the presence of a human in an arena test and their behavioural reactivity to isolation box test as More Active (MA) or Less Active (LA). It was seen that MA animals had higher locomotor activity in the arena and were found to be less fearful in the test arena than LA sheep (Beausoleil *et al.,* 2008, 2012). MA sheep spent more time in close proximity to the human and sniffed the human more often and quicker than LA sheep. In a later study using the same flock (Beausoleil *et al.,* 2012) found consistent inter-flock differences in activity level and several context specific differences in behaviour between MA and LA sheep.

Flocks selected for rearing ability moved less around the arena and bleated less than unselected animals which may indicate that they were less agitated when they were placed in the arena in the absence

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of other sheep (Kilgour, 1998). There were significant flock effects in the sense that a flock with a high average flight speed may not have a high agitation score (Dodd *et al.*, 2014). Hazard *et al.* (2016) found that sheep reared under intensive conditions emitted more high-pitched bleats and had a greater locomotor reactivity than their extensively reared counterparts in response to temporary social isolation, regardless of the presence of a human. When the human was present during the test, lambs reared intensively had a stronger reactivity than lambs reared extensively.

Boissy *et al.* (2005) found that the single lambs emitted fewer low-pitched bleats and attempted to escape the testing arena more frequently than twins. Older lambs were less reactive than younger lambs in the agitation test (Dodd *et al.*, 2014). The youngest lambs spent less time near to both the human and their flock mates in the conflict test and emitted more low-pitched bleats (Boissy *et al.*, 2005). Heavier lambs were more reactive in the behavioural tests conducted by Dodd *et al.* (2014) and the lambs that were the heaviest sniffed the ground more frequently in a study by Boissy *et al.* (2005). In contrast, Pajor *et al.* (2008) found the opposite where the heavier lambs were less reactive and calmer than smaller lambs. Lambe *et al.* (2001) and Wolf *et al.* (2008), however, found no effect of live weight in relation to behaviour in sheep.

Rams appeared less fearful than ewes in a variety of tests. Ewes were more active than rams, as they moved, attempted to escape, emitted high-pitched bleats, and sniffed more frequently while being generally more vigilant (Boissy *et al.,* 2005). Rams on the other hand spent more time near the human in the conflict test and in the corridor test, as reflected by a higher proximity score and a lower avoidance score of an approaching human compared to females (Boissy *et al.,* 2005). Dodd *et al.* (2014) reported similar results to where ewe lambs were more reactive in both the agitation test and the flight speed test (Vandenheede & Bouissou, 1993). Differences between ewes and rams from flocks unselected and flocks selected for rearing ability may reflect the temperament of the animals.

Breed differences can also be found when evaluating temperament of the animal. Terminal sire crossbreds were the most active and tried to escape more frequently than purebreds and their subsequent crosses. When just compared to the purebreds, the crossbreds emitted-more high-pitched bleats (Boissy *et al.*, 2005). Multiparous Dorset Horn ewes were much less active and spent less time near the fence in the test arena than both primiparous and multiparous Sarda ewes in partial mother-lamb separation and isolation tests, while the primiparous Sarda ewes were the most active (Barnard *et al.,* 2015). Romanov lambs tended to emit more low-pitched bleats than Lacaune lambs (Boissy *et al.*, 2005). A study by Wolf *et al.* (2008) examined the behaviour of Scottish Blackface and Hardy Speckled Face ewes by exposure to conflicting motivations. They found that lambs out of Scottish Blackface ewes bleated more, entered more squares, and spent more time distant from the observer in the arena than lambs born from Hardy Speckled Face ewes. It was also observed that there were no major changes in the behaviour exhibited by the animals between the first and second round of the tests, with the behaviours of the third round being just slightly different from those in the first round. Habituation to the test environment and the maintenance of lowered response to the contrived situation for periods of up to one year has been reported in other studies (Kilgour & Szantar-Coddington, 1995; Kilgour, 1998; Goddard *et al.*, 2000)

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A positive correlation was estimated between the behaviour of the lambs and their dams when comparing the time that was spent at the fence (Barnard *et al.,* 2015). Barnard *et al.* (2015) reasoned that the lambs that spent significantly more time at the fence had a higher motivation to reunite with their dams. A significant positive correlation was also estimated between the dams that spent more time away from the fence and their lambs as they seemed to have been challenged with the isolation test away from the flock mates.

In non-restrained tests, the animals being assessed are generally held in a large area and there may be a human in the arena taking either an active or passive role. These tests include the arena test (Murphy *et al.*, 1994; Kilgour & Szantar-Coddington, 1995; Kilgour, 1998; Beausoleil *et al.,* 2008; Wolf *et al.*, 2008; Bickell *et al.,* 2009, Barnard *et al.,* 2015), the conflict test (Boissy *et al.*, 2005), variants of the arena test (Boissy *et al.*, 2005), and the open field test (Kilgour & Szantar-Coddington, 1995; Bickell *et al.,* 2009). Locomotion, vocalisations, feeding, and the latency of animals to approach the human or the feed near the human, are among the measurements commonly recorded in these tests. In tests where the human takes an active role (Boissy *et al.,* 2005) the distance a human can approach the sheep before it retreats is measured. The arena test was originally developed by Fell & Shutt (1989) to quantify the aversion of sheep to humans after surgical stress. However, the animals were initially tested in groups of four, whereas single animals are tested at a time during current arena tests. When testing arena behaviours of lambs in groups it was found that there was little influence of early management in both extensive and semi-extensive systems (Goddard *et al.*, 2000).

In restrained tests the animal is physically restrained in a box. Tests include the isolation box test (Murphy *et al.*, 1994; Beausoleil *et al.,* 2008; Bickell *et al.,* 2009; Plush *et al.*, 2011, Brown *et al.,* 2016), and activity score (Blache & Ferguson, 2005**;** Dodd *et al.*, 2014)**.** The agitation of both ewes and lambs can be measured using an isolation box test. Brown *et al.* (2016) put the sheep individually in a fully enclosed crate for 30 s before the animals were weighed. The number of vibrations that associated with movement and vocalisations are recorded over this period using an agitation meter (Blache & Ferguson, 2005**;** Dodd *et al.*, 2014)**.** An animal which is considered to have a poor temperament will have a high agitation score (Blache & Ferguson, 2005)**.** Reactivity of an animal can also be measured using their flight time, which is measured over a set distance, is recorded in seconds, and is usually measured when the animal leaves a crate. Sheep that have a short flight time, meaning they cover the set distance quickly, is generally regarded as being more fearful and therefore having a poorer temperament than animals with a longer flight time (Blache & Ferguson, 2005)**.**

2.6 Genetics of behaviour and temperament

It was suggested by Faure (1994) that there are three reasons as to why behavioural genetics are not as well studied: (1) Most of the behaviour is seen as being determined mainly by environmental factors, even though the genetic mechanisms inherent to behaviour and performance traits are the same; (2) Behaviour is seen as difficult and labour-intensive to measure, and lastly (3) that measures of behavioural

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traits are not normally distributed. To achieve ongoing genetic gains from selection, there must be additive genetic variation, which is most easily exploited within breeds. However, between breed variation may also be utilised in structured breeding programmes (Brien *et al.*, 2014).

For MBS to be useful as an indirect selection criterion, it must be heritable as well as correlated genetically with survival (Brien *et al.*, 2014). Heritability estimates for MBS ranged from 0.09 to 0.35 (Table 2.1) (Lambe *et al.,* 2001; Everett-Hincks *et al.,* 2005; Brien *et al.,* 2010; Hatcher *et al.,* 2010; Brien *et al.,* 2014; Brown *et al.,* 2016). Repeatability of MBS ranged from low (0.09, Everett-Hincks *et al.,* 2005) to high (0.49, Hatcher *et al.,* 2010). The low repeatabilities suggested that an ewe with a high MBS in one lambing year is unlikely to have a similar MBS in subsequent lambing years. Maternal heritability was estimated as 0.23 (Hatcher *et al.*, 2010). MBS had low genetic variation, as 91% of the total variation is accounted for by environmental effects (Everett-Hincks *et al.,* 2005). Lambe *et al.* (2001) also found that the major sources of variation in MBS were environmental, as the genetic variance for MBS was also small (0.15). They also found a considerable permanent environmental effect of 0.19 on MBS.

Trait	h^2	Reference
MBS	0.13	Lambe *et al.,* 2001
	0.09	Everett-Hincks *et al.,* 2005
	0.20	Hatcher *et al.,* 2010
	0.12	Brien *et al.,* 2010
	0.20	Brown *et al.,* 2016
Lamb survival	0.05	Safari & Fogarty, 2005
	0.01	Villalobos & Garrick, 1999
	0.02	Van Wyk *et al.,* 2003
	0.14	Everett-Hincks *et al.,* 2005
	0.02 - 0.05	Hatcher *et al.,* 2010
	0.014 - 0.010	Brien *et al.,* 2010 (lamb survival to 3d and weaning, respectively)

Lambe *et al.* (2001) reported that the genetic correlation between MBS recorded during different parities were generally high with the phenotypical variance of MBS in each parity being similar and ranging from 1.20 to 1.31. The authors also concluded that MBS was affected by the ewe's sire which indicates that MBS is under genetic control. MBS had low to moderate genetic correlations with production traits, where the correlations between MBS and various bodyweight traits were low but favourable. A favourable moderate genetic correlation were found between MBS and NLW (Brown *et al.*, 2016), where the authors then suggested that selection for MBS can improve lamb survival, particularly in Merinos, where the survival of twins are low.

Table 2.1 Heritability estimates of MBS and lamb survival found in literature

Brien *et al.* (2010) reported that the timed lamb behaviours used in their study (time taken to bleat, - to stand, - to contact the ewe, - to contact the udder, - to follow the ewe) all had low heritability estimates. Only time taken to bleat exhibited a moderate genetic correlation of -0.43 with lamb survival to weaning, indicating that a shorter time taken to bleat is associated genetically with a higher survival rate. For all

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neonatal traits (birth assistance, lamb viability, sucking assistance, and birth weight), Matheson *et al.* (2012) reported that the permanent environmental effect amounted to more of the phenotypic variance, followed by the direct additive genetic effect and the residual variance (50-63 %, 25-38 %, 11-20 % respectively). The heritability estimates in the same study were moderate (birth assistance, 0.26; lamb viability, 0.40; sucking assistance, 0.32) and so were the genetic correlations, which ranged from moderate to high and positive (0.54 to 0.80). Phenotypic correlations were all moderate and positive (0.29 to 0.60) and the standard errors for the heritabilities, genetic and phenotypic correlations were low. Brien *et al.* (2010) reported an estimated heritability of 0.10 for the time taken to stand after birth. Differences between breeds accorded with a contention that early neonatal progress in lambs is partially under genetic control (Slee & Springbett, 1986; Alexander *et al*., 1990; Cloete *et al*., 2002b). Other studies, however, reported no breed differences for early neonatal behaviour (Fahmy *et al*., 1997).

Lamb survival heritability estimates are represented in Table 2.1. The probability of genetic improvement of lamb survival within a breed has been generally regarded as medium to low, as heritability estimates of lamb survival are low (Safari & Fogarty, 2003) (weighted averages of 0.03 for direct heritability and 0.05 for maternal heritability, Safari *et al.*, 2005). Similarly, Lopez-Villalobos & Garrick (1999) reported heritability estimates at 0.01, 0.03, and 0.04 for direct, maternal and total genetic effects, respectively. The heritability of lamb survival was reported as 0.02 in Dormer sheep (Van Wyk *et al.*, 2003). In a study by Sawalha *et al.* (2007) the survival of the lamb after birth was not affected by MBS. Brien *et al.* (2010) reported that the lamb survival traits (to weaning and to 3 days after birth) were low genetically correlated in a favourable direction with MBS. A positive correlation was estimated between direct and maternal genetic effects for lamb viability at birth, suggesting that dams with good genetic mothering ability have a better direct genetic potential to produce lambs with lower mortality rate at birth. However, maternal genetic effects were more important than the direct genetic effects (Sawalha *et al.,* 2007). Riggio *et al.* (2008) reported that the heritability of lamb survival declined with the age of the lamb but was initially moderate, and that lamb survival was positively genetically correlated with the live birth weight of the lamb. Brien *et al.* (2010) reported that the lamb survival traits had a negligible phenotypic correlation with birth weight, and at the genetic level, the correlation between birth weight and lamb survival to 3 days after birth was low and negative (-0.32), and negligible between birth weight and lamb survival to weaning.

Everett-Hincks *et al.* (2005) reported that the direct heritability for lamb survival amounted to 0.14 and the maternal heritability to 0.11. The direct heritability estimates for lamb survival of twins (0.0) differed from that of triplets (0.08). The maternal heritability accordingly amounted to respectively 0.21 and 0.16. Hatcher *et al.* (2010) found much lower heritability estimates for lamb survival for both direct (0.02 to 0.05) and maternal (0.03 to 0.07) effects. The heritability estimates for both lamb survival to 3 days of age and lamb survival to weaning were very low (0.014 and 0.010, respectively) (Brien *et al.,* 2010). Burfening (1993) reported that the estimated variance components for the direct effects on lamb survival were small, and in the same range as the variance components for maternal effects. Hatcher *et al.* (2010) reported that selection for lamb survival will result in little genetic gain as a result of the small proportion of phenotypic variance accounted for by direct genetic, maternal genetic and maternal permanent

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environment components across ages and time periods (1d to 7d and 7d to 110d). Phenotypic and genetic correlations for the direct genetic effects across time periods for lamb survival were all positive and high, as also found for maternal genetic and permanent environmental correlations. Everett-Hincks *et al.* (2005), however, reported that the genetic correlation between the maternal and direct effects for lamb survival were unfavourable at -0.74. Brien *et al.* (2010) reported that lamb survival to weaning and lamb survival to 3 days of age were also very strongly correlated at both phenotypic and genetic level.

In a study by Hatcher *et al.* (2010) the authors concluded that the maternal genetic effects were more important than direct genetic effects for lamb survival, which was consistent with the results of Burfening (1993) and Sawalha *et al.* (2007). The direct genetic variance accounted for the largest variance component relative to the observed phenotypic variation between 7d of age and weaning, indicating that the ability of the lamb to survive on its own is more important than the mothering ability of the ewe.

Litter survival had a low repeatability (Plush *et al.*, 2011) and heritability (Everett-Hincks *et al.,* 2005; Safari *et al.*, 2005; Plush *et al.*, 2011). These results suggest that a dam with a high litter survival in one lambing year is unlikely to have the same litter survival in subsequent years. The main source of variation appeared to be due to the environmental effects, which accounts for 89% of the variation (Everett-Hincks *et al.,* 2005).

Direct selection for ewe rearing ability poses its own set of problems, as it can only be measured for the first time at first lambing while it is not expressed in rams. A possible indirect selection criterion that can solve these two problems is measuring the behaviour of the animals in an arena test. Advantages of the arena test include: 1) it can be measured as early as 6 months of age; 2) the arena behaviour of rams can be measured; 3) potential useful arena behaviour measurements, such as the distance travelled and the number of bleats, are continuously distributed; 4) it is much easier to record than the lambing and rearing performance of individual ewes (Kilgour, 1998). Ewe mothering temperament was moderately to highly heritable (0.35) in a study by Plush *et al.* (2011), which is higher than the estimate of 0.09 reported by Everett-Hincks *et al.* (2005). Ewe mothering temperament showed a positive correlation with litter survival, which had a low heritability (0.09), as well as with agitation score. Agitation score was moderately heritable (0.20) and negatively correlated with flight time and positively correlated with litter survival (Plush *et al.*, 2011). This positive correlation with litter survival indicated that the more agitated the ewe, or the more nervous the temperament, the higher the litter survival is expected to be. The heritability of agitation score found by Plush *et al.* (2011) was lower than the heritability of 0.41 previously derived from a flock divergently selected for it in a study by Blache & Ferguson (2005), which also showed low repeatability for the agitation score. These results for agitation score suggested that selection for this trait will result in little genetic gain, whether it is in the trait itself or for lamb survival (Plush *et al.*, 2011). Low but favourable phenotypic and genetic correlations were reported for ewe mothering temperament and litter survival (Brien *et al.*, 2010; Hatcher *et al.*, 2010; Plush *et al.*, 2011). A positive genetic correlation between mothering temperament and agitation scores indicated that ‘nervous’ ewes produce offspring that display improved maternal scores (Plush *et al.*, 2011). Hazard *et al.* (2016) found that the heritabilities were high for vocalisations, moderate for locomotion and vigilance, and low for the reactivity towards the human.

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Wolf *et al.* (2008) reported that the number of bleats and the number of squares entered were moderately heritable (0.39 and 0.29 respectively) whereas the time spent near the observer and the number of entries into this area also had significantly heritable estimates (0.22). Latency to bleat (0.13) and time spent distant form the observer (0.13) had low to non-significant heritability estimates.

Flight time and the isolation box test exhibited moderate to high heritability estimates in a study by Brown *et al.* (2016). The latter authors showed that flight time was significantly negatively genetically correlated with Maternal Behaviour Score (MBS). From these results it seems as if maternal behaviour and temperament can be improved by selection and that ewes with longer flight times will generally have better maternal behaviour at lambing. Dodd *et al.* (2014), however, found that flight speed and agitation had low to moderate heritability estimates of respectively 0.11 and 0.20, where most of the variance was unexplained. In the same study the behavioural tests were poorly correlated phenotypically and genetically with each other.

The majority of the estimates for measured behaviours indicated a moderate to high repeatability (Kilgour, 1998; Kilgour & Szantar-Coddington, 1995; Wolf *et al.,* 2008). Murphy *et al.* (1994) found high repeatability estimates for all four of the measures they assessed. Measures of vocalisation, locomotion and human-related behaviours in arena tests are more replicable across time and thus are true measures of specific behaviour repertoires in an individual, although habituation may dull these responses.

2.7 The resource population used

There is limited information on the ovine traits discussed under the previous headings in South Africa. However, some of the traits discussed were recorded in one of the local resource populations described by Schoeman *et al.* (2010), namely the Elsenburg Merino flock. Data from this flock was therefore used in this study to quantify the genetics of lamb and ewe behaviour in relation to lamb survival and number of lambs weaned per ewe mated as well as arena behaviour. A brief account of this flock follows:

The Merino Resource project at Elsenburg commenced in 1986 and involved divergent selection for reproduction (expressed as NLW) from a common base flock. The project reported marked divergent responses in reproduction (approximately 2% per annum on the upward direction and 1% per annum in the downward direction – Cloete *et al.,* 2004a). Divergent responses were also reported for live weight and wrinkle score (Cloete *et al.,* 2005b). These responses were in the desired direction in the line selected for improved reproduction. The latter line also exhibited a higher resistance to breech blowfly strike than the line selected against reproduction (Scholtz *et al.,* 2010a). The flock also formed the basis for molecular genetic studies on Random Amplified Polymorphic DNA markers (Naidoo *et al.,* 2005), Cytochrome P450 17α-hydroxylase /17,20-lyase expression on stress management in sheep (Hough *et al.,* 2013, 2015) and SNPs putatively associated with the difference in reproduction rate between the lines (Sandenbergh *et al.,* 2013; Sandenbergh, 2015). Studies on the flock also contributed to a number of postgraduate studies in recent years (Scholtz, 2010; Hough, 2012; Matebesi-Ranthimo, 2015; Sandenbergh, 2015), while also

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contributing to the current study. The research on the flock played a major role in bridging the gap between genotypes and phenotypes in recent years. The flock has been funded by industry for the most of the past two decades and should therefore be considered as one of the most valuable genetic resources in the country. The conservation of this resource population and further selection as well as continued research could thus be seen as a prerequisite. Studies such as the present one contributes to the utility of the flock and provides additional motivation for its continued existence.

2.8 Research questions

This review emphasises the importance in the specific behaviours displayed by both the ewe and the lamb before, during and after parturition that has a putative significant effect on the survival of the lamb. Against this background, the following research questions were addressed:

1. Is neonatal behaviour of lambs heritable, has it been affected by selection in the resource population and are the recorded behaviour traits genetically correlated with lamb survival?
2. Is perinatal behaviour of ewes heritable, has it been affected by selection in the resource population and were the recorded behaviour traits genetically correlated with number of lambs weaned per ewe mated?

The answers to research questions 1 and 2 are complicated by the fact that behaviour repertoires were assessed within lines. Therefore, it is difficult to partition direct and maternal effects, as lamb and ewe effects were confounded within lines. Therefore, a third research question was added, namely:

3. Do lamb behaviour attributes from question 1 hold when lambs are from randomly assigned surrogate ewes in a multiple ovulation and embryo transfer study, and do lambs produced in this way reflect similar levels of performance as reported for the parent lines for production traits of economic importance?

Finally, weaners from the divergently selected flock were subjected to an arena test as described previously. This led to the formulation of a fourth research question, namely:

4. Are arena behaviour traits heritable, has it been affected by selection in the resource population and can it be extrapolated to benefit welfare by improving the handler-animal interface?

The following research chapters set out to explore these research questions in four chapters structured as freestanding documents in scientific paper format.

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Chapter 3
Genetic and environmental (co)variance components and ratios for birth weight, lamb survival and perinatal behaviour traits of Merino lambs

3.1 Abstract

Data for birth weight, lamb survival as well as neonatal behaviour, including lamb records of between 1494 and 2705 lambs were used. The resource population has been divergently selected from the same base population since 1986, either for or against number of lambs weaned per ewe mated and termed the H line or L line respectively. Ram lambs, the progeny of mature ewes and singles were heavier than ewe lambs, the progeny of young and old ewes and multiples respectively. Second multiple had shorter parturitions than singles and first multiples. Ewe lambs and H line progeny experienced shorter parturitions than ram lambs and L line progeny respectively. Sex and selection line had no effect on the latency from birth to suckling, but was affected by the age of the dam, as it decreased as dam age increased. The second multiple took the longest to progress to suckling from the time of birth, with singles being the quickest. H line dams had better maternal cooperation scores than L line dams and maternal cooperation score improved with dam age. Single-trait direct heritability estimates (h^2) in lambs were 0.15 for birth weight, 0.07 for lamb survival, 0.06 for length of parturition, 0.12 for latency from birth to suckling and 0.00±0.03 maternal cooperation score. The inclusion of the maternal genetic variance ratio (m^2) resulted in an improvement in the log likelihood ratio for birth weight, latency of parturition, latency from birth to suckling and maternal cooperation score, yielding estimates of respectively 0.37, 0.18, 0.11 and 0.07. Maternal permanent environment variance ratios (c^2) were 0.07 for lamb survival and 0.17 for maternal cooperation score. Lamb survival was favourably correlated to latency of parturition and maternal cooperation score on the genetic level. Based on the genetic parameters it was evident that neonatal behaviour was, to an extent, under genetic control in paddock-reared sheep. Selection for traits such as these could benefit animal welfare.

3.2 Introduction

Lamb mortality is a major constraint to efficient sheep production (Haughey 1991; Brien et al., 2014), as it remains a significant welfare and production issue in the industry (Matheson *et al.*, 2011). Lamb mortality has been found to be highest within the first 3 days of postnatal life (Cloete, 1992; Nowak *et al.*, 2000; Sawalha *et al.*, 2007; Brien *et al.,* 2010). Aberrant ewe behaviour has been recognised as one of several significant causes of lamb mortality (Alexander, 1960; 1988; Arnold & Morgan, 1975). Another significant causes identified has been birth weight of lambs (Gama *et al.,* 1991). The bond between the ewe and the lamb is essential for lamb survival as it facilitates the early suckling behaviours and the intake of colostrum (Nowak *et al.*, 2000). In intensive systems, the trend is to increase the productivity, among others by increasing litter size. However an increase in litter size has led to increased lamb mortality which

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can not entirely be because of birth weight of the lamb (Kouimtzis, 1986; Knight *et al.*, 1988; Lindsay *et al.,* 1988). Both ewes and lambs contribute to survival of the lamb. The ewe must have the ability to give birth without complications to a live lamb and provide adequate mothering, a stable colostrum and milk supply, whereas the lamb must adapt outside of the uterine environment, and must be able to stand and suckle quickly (Brien *et al.,* 2014). Selective breeding has been advocated as a means of improving lamb survival and ewe rearing ability under paddock conditions (Le Neindre & Poindron, 1990; Lindsay *et al*. 1990; Haughey, 1991). The ewe's quality of maternal care given to her lamb(s) can, in part, affect the survival of the lamb (Bickell *et al.,* 2011). Selection for number of lambs weaned per ewe mated (NLW) has previously resulted in improved maternal and lamb behaviour that favours the survival of the lamb (Cloete *et al.*, 2005b). Behavioural adaptations were contended to contribute to selection responses in lamb survival in the pastoral areas (Alexander, 1988). Genotype of the lamb only influenced the early behavioural development in the immediate postnatal period, but after this the ewe had a significant effect on the frequency of the expression of the behaviours (Dwyer & Lawrence, 2000). Genetic (co)variances for lambing and neonatal behaviour of South African dual-purpose breeds were reported more than a decade ago (Cloete *et al*., 2002a), in which low to moderate levels of direct and maternal genetic variation were found. Nowak (1996) indicated that there are two approaches to control mortality of lambs due to behavioural factors: 1) improve the physical conditions at and around lambing to ensure maximal survival, even if optimal maternal care is not provided, and 2) is to improve the early mother-young relationship, to ensure that the lamb has the best chance of survival even when circumstances are difficult.

Lamb survival, particularly that of multiples, were improved in the H line which was selected for NLW, for the period from 1993 to 1997 (Cloete & Scholtz, 1998) and from 1998 to 2001 (Cloete *et al*., 2002b). This study contributes to a better understanding of factors associated with lamb survival, and possible mechanisms that are involved in the genetic improvement of rearing ability in pasture-fed ewes. It provides a basis for the further extension of contentions by several authors (see Lindsay *et al*., 1990; Haughey, 1991; Le Neindre *et al*., 1998) that the ultimate objective in pastoral systems should be the establishment of genotypes that are capable of rearing their offspring with minimal external inputs. Against this background, data of a resource flock divergently selected for NLW were analysed to obtain line effects as well as genetic parameters and trends for lambing behaviour traits. Genetic correlations were derived at the same time with lamb survival and birth weight.

3.3 Materials and Methods

3.3.1 Animals and location

Using maternal ranking values for lambs reared per joining, two lines of Merino sheep were divergently selected from the same base population since 1986. The details for the selection procedure of replacements can be found in the literature (Cloete *et al.,* 2004b; 2009) and the research conducted on the resource flock was covered in Chapter 2. Replacements for the High (H) line were preferred as the ewe and ram progeny of ewes that reared more than one lamb per joining (i.e. reared twins at least once).

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In contrast, the Low (L) line replacements were from ewes that reared less than one lamb per joining (i.e. were barren or lost all lambs, at least once). In recent years, selection have been aided by breeding values derived from a single-trait, repeated-records animal model as described by Cloete *et al.* (2009).

The two lines were maintained as a single flock since establishment except during joining in single sire groups to 4-5 rams. Irrigated kikuyu (*Pennisetium clandestinum)* paddocks were used during joining in the summer (January – February) and during lambing in the winter (June – July) for the duration of this experiment. There were 10 kikuyu paddocks on which lambing took place of approximately 0.3 to 0.5 ha each, as described by Cloete & Scholtz (1998) and Cloete *et al.* (2002b) For the remainder of the year, dryland lucerne *(Medicago sativa)* and medic *(M. truncatula)* pastures were used. Occasionally during winter, an oat *(Avena sativa)* fodder crop was used, whilst oat crop residues and standing hay were sometimes available during spring and early summer. Supplementary feeding was rarely needed as nutrition was mostly adequate for the duration of the year. Ewes were shorn within 3-4 weeks before the commencement of lambing annually. This study was conducted over a 10-year period from 1993 to 2002.

3.3.2 Observations and recordings

During peak lambing (approximately 3 weeks) the lambing flocks were continuously observed by one to two trained observers. The ewes quickly adapted to the presence of the observers and could be approached closely (<10m) without unnecessary disturbance. Five lambing paddocks were utilised during the day and another five floodlit paddocks were utilised at night (Cloete & Scholtz, 1998). One behavioural trait that was recorded included length of parturition of individual lambs, which was calculated as the period between the first definite sign of parturition and the birth of that specific lamb. The birth of the previous lamb was regarded as the beginning of parturition for subsequent litter mates in multiple births. Assistance was given to ewes during birth which was experiencing difficulty to lamb and had not yet delivered within 3.5 hours after the commencement of parturition (Cloete *et al.,* 2002a). However, a few ewes were assisted if they had not delivered 2 hours after a body part of the lamb was presented at the vulva or 30 minutes after obvious malpresentation of clearly recognizable body parts (Cloete & Scholtz, 1998). The time prior to assistance was accepted as the length of parturition in such cases. Another behavioural observation on lambs included the time from standing to first suckling for at least 10 seconds, which describes the latency from standing to suckling. Lambs were identified by a stock marker spray, after being groomed by their dams for at least 15 minutes, which was according to a unique number applied to the sides of their dams with stockmarker spray. Multiple lambs were also identified according to order of birth. Within 24 hours of the birth of the individual lambs, birth weight was recorded to the nearest 0.1kg (Cloete & Scholtz, 1998). Ewe behaviour that were recorded during suckling attempts were based on ewes that frustrated the first attempts of the neonate to suckle and included backing, circling, and/or butting (Alexander, 1988; Cloete & Scholtz, 1998). The behaviours that aided in the neonates first suckling attempts were also noted, which included standing still, adopting a slight hunched posture to enable access to udder, and nudging the lamb in a position that will facilitate suckling (Cloete & Scholtz, 1998). These recordings were accumulated to give the ewe an overall maternal co-operation score on a 6-point scale, where a positive score was given

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for each of the distinct behaviour repertoires that aided neonates' suckling attempts, and similarly a negative score was given for each distinct behaviour that hampered neonates' suckling attempts (Cloete & Scholtz, 1998).

3.3.3 Statistical analysis

Latency traits (the intervals between the commencement of birth and the expulsion of the lamb as well as from birth to apparently suckling) did not conform to the conventions needed for analysis of variance. The distributions of these traits were normalised by transformation to natural logarithms after 3 were added to account for very short time intervals. ASREML was used to estimate fixed effects and derive variance components for birth weight and the behavioural traits in single-trait analyses (Gilmour *et al.,* 1999). The use of a linear model to analyse binomial data needs some comment. Preliminary results reported by Cloete et al. (2014) suggested that threshold-trait analyses yielded the same conclusions. However, these results were on the underlying liability scale and thus more difficult to interpret. The fixed effects that were considered for the lambs were year of birth (1993-2002), selection line (H line and L line), sex (ram or ewe), age of the dam (2-7+ years),birth type (single or multiple) for birth weight, lamb survival and birth order (single, first multiple or second multiple) for the behavioural traits. For the first analyses, various combinations of fixed effects were fitted including interactions among them to obtain an operational model. The effects that were found to be significant ($P < 0.05$) in these preliminary analyses were retained for subsequent analyses. Random terms were then added to the operational model, resulting in the following genetic models for analyses (in matrix notation):

$y = Xb + Z_1a + e$ (1)

$y = Xb + Z_1a + Z_3m + e$ (2)

[Correlation (a,m)=0]

$y = Xb + Z_1a + Z_2c + e$ (3)

$y = Xb + Z_1a + Z_3m + Z_2c + e$ (4)

[Correlation (a,m)=0]

$y = Xb + Z_1a + Z_3m + e$ (5)

[Correlation (a,m)=$A\sigma_{am}$]

$y = Xb + Z_1a + Z_3m + Z_2c + e$ (6)

[Correlation (a,m)= $A\sigma_{am}$]

In these analyses, y was a vector of observations for lamb birth weight, survival or behavioural traits, b, a, m and c were vectors of fixed effects, direct genetic variances, maternal genetic variances and maternal permanent environmental variances respectively, X, Z_1, Z_2, and Z_3 were the corresponding incidence matrices relating the respective effects to y, e was the vector of residuals, A was the numerator relationship matrix, and σ_{am} was the covariance between direct genetic and maternal effects.

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It was assumed that:

$V(a) = A\sigma^2_a$; $V(m) = A\sigma^2_m$; $V(c) = I\sigma^2_c$; $V(e) = I\sigma^2_e$,

With I being an identity matrix; σ^2_a, σ^2_m, σ^2_c and σ^2_e direct genetic variance, maternal genetic variance (as a trait of the lamb) and the maternal permanent environmental variance and environmental (residual) variance respectively. It should be noted that direct genetic variances were fitted by default even for traits like maternal cooperation score where it was not necessarily significant initially. This was done to ensure that genetic correlation could be derived between all traits. All analyses included the full pedigree file, consisting of 4351 individuals, the progeny of 216 sires and 1107 dams.

Log likelihood tests were conducted to determine the most suitable model for each trait in single-trait analyses. The random effects models that best fitted the data for each trait were identified, using the log likelihood ratios obtained from the respective analyses (Snyman *et al*., 1995). Subsequently, 2-trait animal models were fitted. These analyses allowed the calculation of all relevant direct and maternal genetic correlations between traits, as well as dam permanent environmental correlations, all together with their appropriate standard errors.

The direct and maternal breeding values for traits were obtained and averaged within birth years for lambs. Genetic trends were inspected for divergence between the lines, using the standard errors that were obtained for the regression coefficients. The breeding values used for this were obtained from analyses where selection line and its interactions with other traits were excluded from the operational model. Including the selection line as fixed would reduce the breeding values for genetic differences between lines that accrued as a result of selection. Breeding values were on the transformed scale in the case of latency traits involving time intervals. To report results on the normal scale, the overall phenotypic mean was added to the averaged breeding values prior to transformation back to the normal scale. These averaged breeding values on the normal scale were then expressed as deviations from the overall phenotypic mean prior to transformation.

3.4 Results

3.4.1 Descriptive statistics

The descriptive statistics for birth weight, length of parturition, latency from standing to suckling and maternal cooperation score is given in Table 3.1. Coefficients of variation ranged from 16.3% for the transformed latency from birth to suckling and 60.3% for lamb survival. Overall, it was evident that sufficient phenotypic variation was available to support genetic progress in all traits, should it be desired.

Table 3.1 Descriptive statistics for birth weight, length of parturition, latency from standing to suckling, and maternal cooperation score

Statistic	Trait

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	Birth Weight (kg)	Lamb survival	Length of Parturition (Minutes)	Standing to suckling (Minutes)	Maternal cooperation score
Number of observations	2705	2705	1620	1494	1494
Mean±SD	3.98±0.83	0.73±0.44	3.50±0.94	4.10±0.67	5.29±0.99
CV (%)	20.8	60.3	26.9	16.3	18.7
Range	1-7	0-1	1.4-6.8	1.8-6.8	1-6
Skewness	-0.00	-1.06	0.31	0.39	-1.63
Kurtosis	-0.33	-0.87	-0.30	0.18	2.64

3.4.2 Fixed effects

Selection line affected all traits but for the latency from birth to first apparently suckling. Year effects were present for all traits (Table 3.2). We did not discuss these in detail, as years depend on a combination of management and climatic effects which are not repeatable. The effects were kept in analyses for the variance it controlled. Sex affected all traits except for the latency from birth to first apparently suckling, while all traits were dependent on the age of dam except for length of parturition. Birth type or birth order affected all traits but maternal cooperation score. Selection line interacted with birth type for lamb survival and with age of dam for length of parturition. The latency from birth to first apparently suckling was related to maternal cooperation score.

Table 3.2 Significance of fixed effects fitted for birth weight, length of parturition, latency from standing to suckling, and maternal cooperation score

Fixed effect	Trait				
	Birth Weight (kg)	Lamb survival	Length of Parturition (Minutes)	Standing to suckling (Minutes)	Maternal cooperation score
Selection line (SL)	*	**	*	0.44	**
Year	**	**	*	**	**
SL x Year	0.09	0.54	0.16	0.21	0.89
Sex	**	*	*	0.10	*
Age of dam	**	**	0.10	**	**
SL x Age of dam	0.21	0.20	*	0.09	0.17
Birth type	**	**	-	-	-
SL x Birth type	0.70	**	-	-	-
Birth order	-	-	**	**	0.11
SL x Birth order	-	-	0.06	0.99	0.58
Cooperation	-	-	-	**	-

* Significant ($p<0.05$); ** Significant ($p<0.01$); Actual significance for $p>0.05$

Birth weight, length of parturition, the latency from birth to first standing for > 10 seconds, the latency from birth to first suckling, the latency from standing to first apparently suckling and maternal cooperation score are given in Table 3.3. H line lambs were slightly heavier with a markedly better overall survival than L line lambs ($p<0.05$; Table 3.3).

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Table 3.3 Least squares means for traits measured (±s.e). Back transformed means of traits that were analysed after transformation to natural logarithms are given in parentheses.

Fixed effects	Birth Weight (kg)	Lamb survival	Length Of Parturition (Minutes)	Standing to suckling (Minutes)	Maternal cooperation score
Selection Line	*	**	*	n.s.	**
H Line	4.06±0.06	0.76±0.03	3.42±0.05 (27.6)	4.04±0.06 (53.9)	5.40±0.05
L Line	3.93±0.08	0.63±0.03	3.69±0.07 (38.9)	4.11±0.07 (57.8)	5.08±0.08
Birth Type or Order	**	**	**	n.s	n.s
Single	4.49±0.06	0.78±0.02	3.81±0.05 (42.0)	3.95±0.05 (49.1)	5.31±0.05
Multiple/First Multiple	3.50±0.06	0.60±0.02	3.72±0.06 (38.4)	4.12±0.06 (58.6)	5.18±0.06
Second Multiple	-	-	3.13±0.06 (19.9)	4.15±0.06 (60.6)	5.21±0.07
Sex	**	*	*	n.s.	*
Ram	4.14±0.06	0.67±0.02	3.63±0.05 (34.6)	4.10±0.05 (57.3)	5.19±0.05
Ewe	3.86±0.06	0.72±0.02	3.48±0.05 (29.5)	4.05±0.05 (54.4)	5.29±0.05
Age of Dam (Years)	**	**	n.s.	**	**
2	3.72±0.08	0.66±0.03	3.71±0.08 (37.4)	4.25±0.07 (67.2)	4.43±0.08
3	3.97±0.07	0.74±0.03	3.56±0.07 (32.2)	4.11±0.06 (58.1)	5.17±0.07
4	4.07±0.07	0.76±0.03	3.48±0.06 (29.5)	4.03±0.06 (53.1)	5.43±0.07
5	4.14±0.07	0.74±0.03	3.53±0.06 (31.2)	4.07±0.06 (55.8)	5.44±0.07
6	4.11±0.07	0.68±0.03	3.41±0.06 (27.2)	3.98±0.06 (50.4)	5.40±0.07
7	3.97±0.10	0.60±0.04	3.63±0.08 (34.8)	4.01±0.10 (52.0)	5.55±0.15
Cooperation	-	-	-	0.145±0.017	-

n.s. – Not significant (p>0.05); * - Significant (p<0.05); ** Significant (p<0.01); - No mean available

Lamb survival of the respective lines varied with birth type, as depicted by the interaction of selection line with birth type (Figure 3.1).

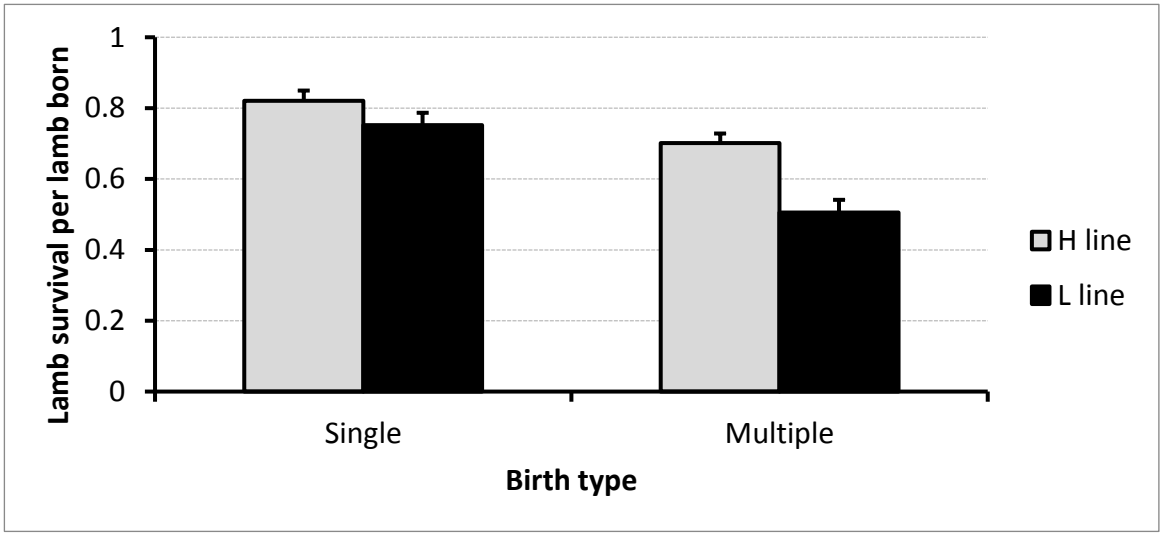

Figure 3.1 Depicting the interaction (± s.e.) between selection line and birth type for overall lamb survival

The survival of single H line lambs was not significantly improved compared to L line contemporaries (p>0.05). However, multiple H line lambs had a 39% higher survival than their counterparts in the L line.

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Ram lambs were heavier than ewes, singles were heavier than multiples and progeny of mature ewes were heavier than lambs born to 2-year-old ewes (all p<0.01).

Overall, H line lambs experienced shorter births than L line lambs (p<0.05). This line difference was complicated by interactions of selection line with birth order (p=0.06; Figure 3.2) and age of dam (p<0.05; Figure 3.3).

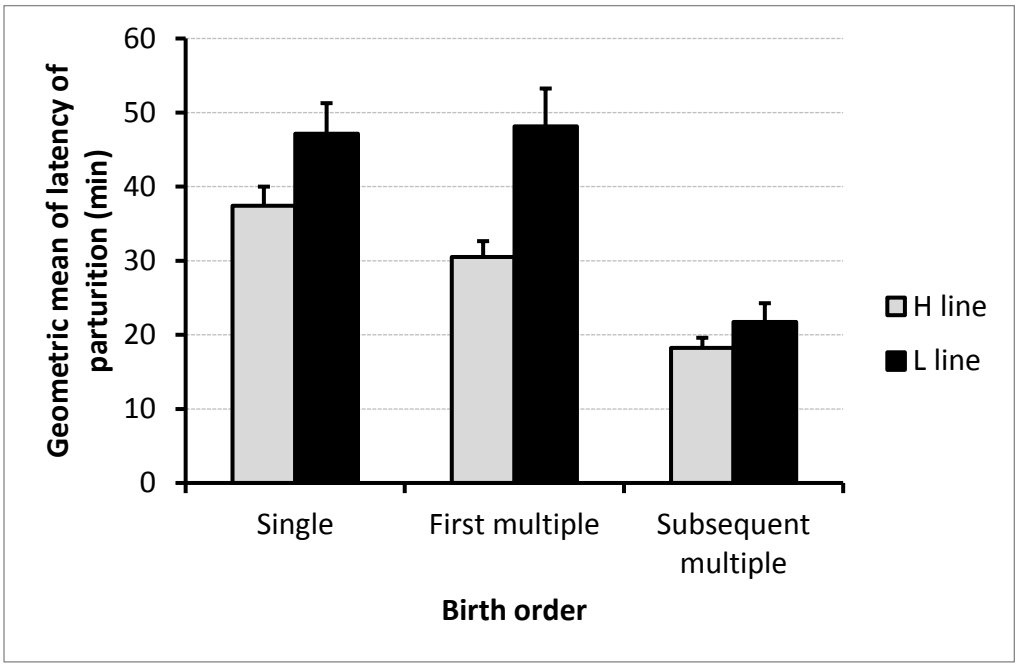

Figure 3.2 Depicting the interaction (± s.e.) between selection line and birth order based on geometric means for the latency of parturition

Although the former interaction only approached significance, it was of interest to note that first multiples had shorter parturitions than singles in the H line but not in the L line.

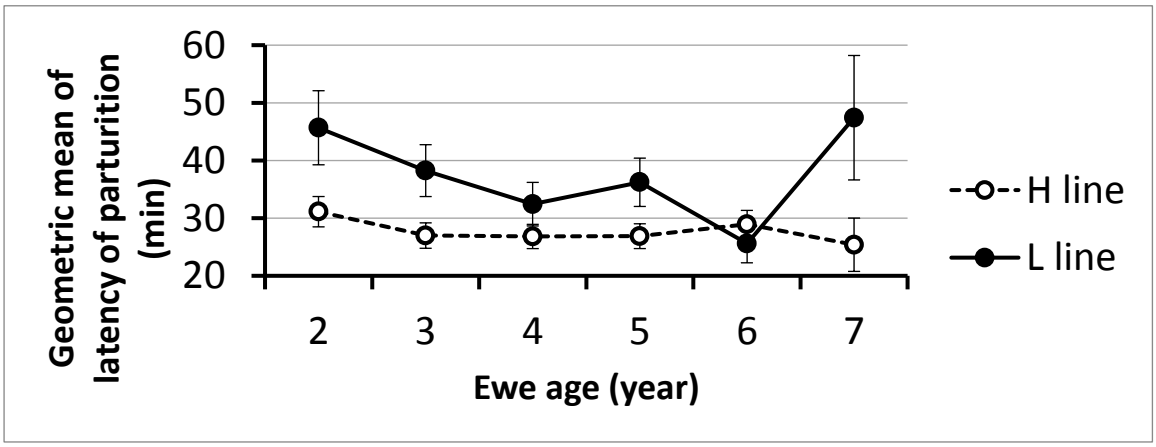

Figure 3.3 Depicting the interactions (of selection line with birth order and age of dam

Subsequent multiples had shorter parturitions than first multiples and singles in both lines, with no apparent line difference (p>0.05).

The length of parturition of L line lambs were 26% longer in singles and 59% longer in first multiples when compared relative to their H line contemporaries.

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H line progeny had shorter length of parturition than their L line contemporaries, except for the ewe age group of 6 years, resulting in the significant selection line x birth type interaction (Figure 3.3).

Ewe lambs had shorter births, an improved lamb survival and a slight advantage in terms of dam cooperation score compared to ram lambs ($p<0.05$). Overall, subsequent multiples had the shortest parturition time compared to both first multiples and singles (Table 3.3). The latency to suckle after birth decreased with the age of the dam ($p<0.01$), with the shortest latencies between 4 – 6 year of age, thereafter, increasing again in 7+-year-old ewes.

Table 3.4 Log likelihood ratios for birth weight and the behavioural traits in Merino lambs of the H and L lines under seven different models of analysis. The "best' models are denoted in bold and italic font

	Trait				
Model Fitted	Birth Weight (kg)	Lamb survival	Length of Parturition (Minutes)	Standing to suckling (Minutes)	Maternal cooperation score
Fixed effects only	-379.278	858.250	-648.004	-66.110	-709.924
+ h^2	-221.442	872.901	-638.350	-48.750	-691.588
+ h^2 + m^2	***-78.977***	876.005	-626.669	***-42.308***	-671.790
+ h^2 + c^2	-111.322	***878.582***	-628.255	-44.215	-688.116
+ h^2 + m^2 + c^2	-78.740	878.710	-625.981	-42.148	***-666.866***
+ h^2 + m^2 + r_{am}	-78.968	876.060	***-624.676***	-41.480	-671.765
+ h^2 + m^2 + c^2 + r_{am}	-78.733	878.975	-624.055	-41.362	-666.707

Co) variance ratios: h^2 = direct additive; m^2 = maternal additive; c^2 = maternal permanent environment; r_{am} = genetic correlation between direct and maternal effects

Single lambs were the quickest to progress to suckling after birth ($p<0.01$), followed by first multiples and second multiples with no significant difference between the latter groups ($p>0.05$; Table 3.3).

Birth order did not influence maternal cooperation score ($p>0.01$). H line lambs experienced higher levels of maternal cooperation score than their contemporaries in the L line ($p<0.01$). Maternal cooperation scores also improved with dam age ($p<0.01$), with little difference between the ages of 4- to 7+-years of age.

3.4.3 Random effects

In analyses of the lamb data, the model that fitted the data the best for birth weight included direct additive and maternal additive effects (Table 3.4). Random effects for lamb survival included direct additive and dam permanent environmental effects. The inclusion of both direct and maternal additive effects, as well as their covariance fitted the data best for length of parturition. The latency from birth to suckling included direct and maternal additive effects. Maternal cooperation score was analysed with a model including direct and maternal additive effects as well as maternal permanent environmental effects

Direct heritability (h^2) estimates amounted to 0.15 for birth weight, 0.07 for lamb survival, 0.06 for the latency from the commencement of parturition to the expulsion of the lamb, 0.12 for the latency from birth to apparently suckling and 0.00 for maternal cooperation score (Table 3.5). All estimates but for length of parturition and maternal cooperation score were more than twice the corresponding standard errors.

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Significant ($p<0.05$) maternal additive variance ratios (m^2) were 0.37 for birth weight, 0.18 for length of parturition, 0.11 for latency form birth to suckling and 0.07 for maternal cooperation score. The dam permanent environmental variance ratios (c^2) were 0.07 for lamb survival and 0.17 for maternal cooperation score. The genetic correlation between direct and maternal effect for the latency from the commencement of lambing to expulsion was -0.78. The estimate of 0.00 for maternal cooperation score indicated that this trait was primarily an ewe trait, expressed on the maternal level. Lambs did not seem to have a level of control on the expression of this behaviour by their dams. Because of this, the direct genetic effect of maternal cooperation score was not modelled in the two-trait analyses.

Two-trait heritability estimates were within 0.01 of corresponding single-trait estimates when averaged across analyses (Table 3.6). Two-trait maternal heritability estimates were accordingly within 0.02 of the corresponding single-trait analyses. It was notable that some additive variation repartitioned to h^2 (0.09) from m^2 (0.16) for length of parturition when analysed with lamb survival. Maternal Cooperation score was affected accordingly when analysed with lamb survival, h^2 and m^2 estimates amounting to respectively 0.06 and 0.05

Table 3.5 Single-trait (co)variance component estimates (±s.e.) for birth weight, lamb survival and the respective behavioural traits in Merino lambs of the H and L lines

(Co)variance components and ratios	Trait				
	Birth Weight (kg)	Lamb survival	Length of Parturition (Minutes)	Standing to suckling (Minutes)	Maternal cooperation score
(Co)variance components					
Direct additive (σ^2_a)	0.0769	0.0123	0.0435	0.0439	0.0027
Maternal genetic (σ^2_m)	0.1866	–	0.1385	0.0429	0.0611
Maternal PE (σ^2_c)	–	0.0120	–	–	0.1537
Direct-maternal correlation (σ_{am})	–	–	-0.0606	–	–
Residual (σ^2_e)	0.2370	0.1593	0.6419	0.2873	0.6710
Total phenotype (σ^2_p)	0.5006	0.1836	0.7633	0.3741	0.8885
(Co)variance ratios					
h^2	0.15±0.04	0.07±0.03	0.06±0.04	0.12±0.06	0.00±0.03
m^2	0.37±0.03	–	0.18±0.05	0.11±0.03	0.07±0.05
c^2	–	0.07±0.02	–	–	0.17±0.05
r_{am}	–	–	-0.78±0.26	–	–

– Effect not significant according to log likelihood ratio; (Co) variance ratios: h^2 = direct additive; m^2 = maternal additive; c^2= maternal permanent environment; r_{am} = genetic correlation between direct and maternal effects

Genetic correlations indicated that lambs heavier at birth would also have longer parturitions, that lambs with shorter parturitions would also have higher lamb survival rates and that lambs with longer parturitions would also take longer to progress from of birth to suckling (Table 3.6). Phenotypic correlations resembled genetic correlations in sign but were mostly smaller in magnitude. Maternal genetic correlations also indicated that heavier lambs at birth would have longer parturitions while lambs with longer parturitions would also progress slower from birth to first apparently suckling. The standard errors for phenotypic correlations were relatively low, indicating that they were fairly reliable.

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Table 3.6 Two-trait (co)variance component estimates (±s.e.) for birth weight, lamb survival and the respective behavioural traits in Merino lambs of the H and L lines

Variance component and trait	Trait				
	Birth Weight (kg)	Lamb survival	Length of Parturition (Minutes)	Standing to suckling (Minutes)	Dam cooperation score
Components					
Direct additive (σ^2_a)	0.0801	0.0119	0.0601	0.0474	–
Maternal genetic (σ^2_m)	0.1901	–	0.1408	0.0436	0.0714
Maternal PE (σ^2_c)	–	0.0118	–	–	0.1465
Direct-maternal correlation (σ_{am})	–	–	-0.0621	–	–
Residual (σ^2_e)	0.2355	0.1594	0.6971	0.2881	0.6736
Total phenotype (σ^2_p)	0.5057	0.1837	0.8154	0.3742	0.8857
h^2 in bold on the diagonal, r_G below and r_P above the diagonal*					
Birth Weight	***0.16±0.04***	0.15±0.02	0.26±0.03	-0.06±0.03	0.06±0.03
Lamb survival	0.13±0.24	***0.06±0.03***	-0.09±0.03	-0.03±0.03	0.04±0.03
Length of Parturition	0.82±0.27	-0.69±0.25	***0.07±0.03***	0.15±0.03	0.01±0.03
Standing to suckling	0.13±0.25	-0.28±0.30	0.62±0.25	***0.13±0.06***	-0.21±0.03
m^2 in bold on the diagonal, r_M below the diagonal*					
Birth Weight	***0.38±0.03***	–	–	–	–
Length of Parturition	0.33±0.11	–	***0.16±0.04***	–	–
Standing to suckling	-0.02±0.14	–	0.51±0.25	***0.11±0.06***	–
Dam cooperation score	-0.01±0.17	–	0.02±0.18	-0.38±0.15	***0.08±0.05***
c^2 in bold on the diagonal, r_{PE} below the diagonal*					
Lamb survival	–	***0.07±0.02***	–	–	–
Maternal cooperation score	–	0.07±0.17	–	–	***0.16±0.05***

*r_G – genetic correlation; r_M – maternal genetic correlation; r_{PE} – dam permanent environmental correlation; r_P – phenotypic correlation

3.4.4 Predicted breeding values and genetic trends

Predicted breeding values were only considered for the behavioural traits, as birth weight were reported on by Cloete et al. (2003b) and lamb survival by Cloete et al. (2009). Maternal cooperation score had limited genetic variation (Table 3.4) and was thus also not considered. Averaged direct breeding values (±s.e.) for length of parturition on the transformed scale (with values on the observed scale in brackets) amounted to -0.044±0.002 (-1.63) min for the H line and 0.017±0.003 (0.47) min for the L line. Corresponding values for the latency from birth to apparently suckling were -0.005±0.002 (-0.54) and 0.047±0.003 (2.53) min respectively. Maternal breeding values for length of parturition were accordingly -0.070±0.003 (-2.48) min for the H line and 0.069±0.005 (2.34) min for the L line. The corresponding values for the latency from birth to standing were respectively -0.004±0.002 (-0.49) and 0.041±0.002 (2.17) min. It is thus evident that the H line was genetically geared for shorter parturitions and quicker progress towards suckling for the first time.

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Regression analyses indicated a significant genetic trend for maternal breeding values for length of parturition, amounting to -0.308±0.128 min on the observed scale in the H line (Figure 3.4). The slope of the corresponding genetic trend in the L line amounted to 0.191±0.247 min (p=0.46).

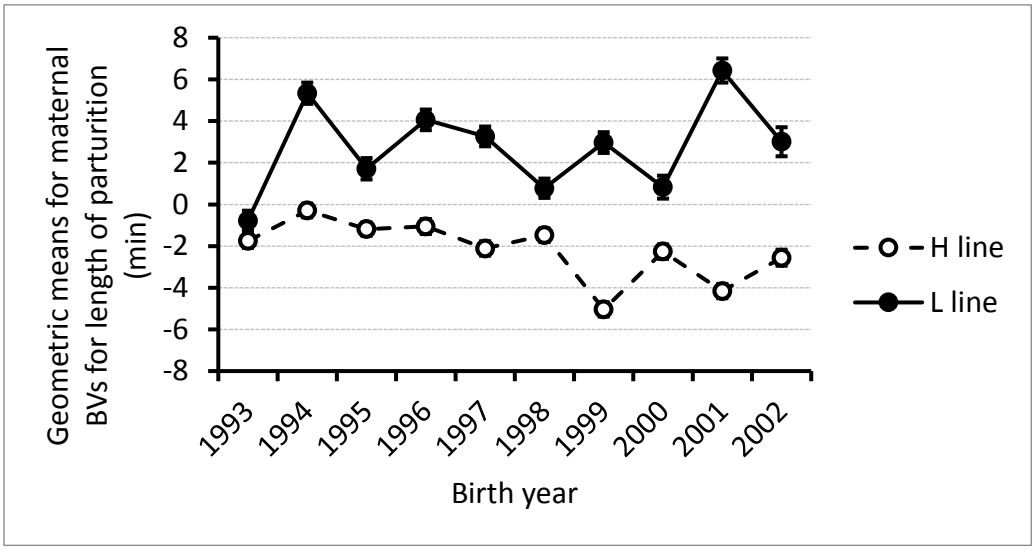

Figure 3.4 Means (±SE) depicting genetic trends as geometric means for the latency of parturition based on maternal genetic breeding values in the H and L lines.

3.5 Discussion

3.5.1 Descriptive statistics

Lamb survival to weaning had a coefficient of variation (CV) of 49.4% - 50% (Brien *et al.,* 2010*,* 2014) which is similar to the estimate found in this study. CV for birth weight were 14.2 for wool breeds, 16.5 for dual-purpose breeds, and 19.2 for meat breeds (Safari *et al.,* 2005).

3.5.2 Fixed effects

The birth weight of the lambs was independent of birth order and maternal cooperation score, but was significantly influenced by selection line, sex of the lamb, age of the dam and birth type. H line lambs were somewhat heavier at birth than L line lambs with a substantially better survival. The effect of gender, birth type, and age of dam on birth weight is in accordance with Knight *et al.* (1988) and Dixit *et al.* (2001). Smaller lambs are reported to be born more from primiparous ewes than multiparous ewes (Hight & Jury, 1970; Cloete, 1992; Dwyer, 2003). Ram lambs were heavier at birth than ewe lambs, which is consistent with literature (Hight & Jury, 1970; Dwyer & Lawrence, 1998; 1999a; Dixit *et al.*, 2001; Dwyer *et al.,* 2005; Everett-Hincks & Dodds, 2008), and ram lambs were also reported to have longer parturitions than ewe lambs (Dwyer, 2003). Owens *et al.* (1985) however, only saw a sex effect when the lambs were born as singles. Single lambs were heavier at birth than lambs from larger litters (Owens *et al.,* 1985; Dixit *et al.,* 2001; Dwyer *et al.,* 2005), as some report a 1 kg decrease in birth weight for each increase in lamb number (Dwyer *et al.,* 2005). Birth order was also found not to be of significance in a study by Owens *et al.* (1985) which is in accordance with the results found in this study.

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H line lambs similarly had shorter parturitions, most evidently so in first multiples. The parity of the ewe did not have an effect on the length of parturition (Cloete, 1992; Dwyer, 2003; Cloete *et al.,* 2002c) and that the environment of the ewe did not affect length of parturition either (Arnold & Morgan, 1975). Alexander *et al*. (1993) found no effect of parity on length of parturition in progeny of ewes > 2 years of age. They did, however, report that length of parturition was longer in progeny of 2-year old maiden ewes than in older dams. Younger ewes have also been found to require more assistance at lambing than older ewes (Dwyer & Bünger 2012).

Second-born lambs in multiple litters had shorter parturitions than either single or first-born lamb in multiple litters, which is also seen in literature (Haughey, 1991; Dwyer & Lawrence, 1998). Owens *et al.* (1985) reported that second-born twin appeared at the vulva quicker than first-born twins. Similar results in the literature (Arnold & Morgan 1975; Cloete *et al*. 2002c) were related to dilation of the cervix, facilitating the delivery of subsequent lambs (Arnold & Morgan 1975). Single lambs had shorter parturitions than twins (Owens *et al.,* 1985; Cloete *et al.,* 1992), and triplets had the longest length of parturitions (Cloete, 1992), which does not accord with the results of this study.

Assistance at birth was required more by lambs born as singles (Dwyer & Bünger; 2012; Matheson *et al.,* 2012). Ram lambs had a longer (p<0.05) parturition time than ewes. This result accords with previous results in other flocks (Alexander *et al*. 1993; Cloete *et al*. 2002*c*; Dwyer 2003). Dwyer (2003) found that the longer parturition time of ram lambs could not be explained by birth weight only, but ram lambs also required more assistance at birth than ewe lambs (Dwyer & Bünger 2012, Matheson *et al.* 2012).

It is notable that Cloete & Scholtz (1998) also reported that the better survival of H line lambs was largely driven by an improved/increased survival of multiples. Atkins (1980) similarly reported a better lamb survival in the Trangie Fertility flock despite a higher birth rate. H line lambs similarly had shorter parturitions, most evidently so in first multiples. Whether this could be related to the improved survival of H line lambs is unknown at this stage but worthy of further study. No evident clarification can be provided for the interaction of selection line with ewe age, which was also seen in Chapter 4 in the ewe data. This response was unexpected and not readily explicable. As it primarily involved a single age group, it might be due to coincidence. In a study involving Marshall Romney (selected for rearing ability) and Control Romney lines in New Zealand, parturient deaths were found to be higher in lambs sired by Control Romney rams than in those lambs sired by Marshall Romney rams (Knight *et al*. 1988). This effect was, however, more pronounced in the Control Romney ram by Marshall Romney ewe combination.

The age of the dam was significant in its effect on the latency from standing to suckling, as lambs had shorter latencies as the age of the ewe increased. Lambs of maiden ewes took a longer time to progress from standing to suckling than lambs cared for by mature ewes in the literature (Slee & Springbett, 1986; Cloete & Scholtz, 1998; Cloete *et al*., 2002c), whereas lambs from older ewes took longer to suck than younger ewes in a study by Matheson *et al.* (2012). Ram lambs were significantly slower than ewes to seek the udder, to suck (Dwyer & Lawrence, 1998; Dwyer, 2003; Dwyer *et al.,* 2005), and from standing to sucking (Alexander *et al.,* 1993). No significant differences between ram and ewe lambs were found in

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this study. Triplet lambs took longer to suck (Cloete *et al.,* 2002c) and had a longer latency from standing to sucking than twins or singles (Owens *et al.,* 1985; Dwyer & Morgan, 2006). Litter size or birth order had no significant effect on early neonatal behaviours (Dwyer & Lawrence, 1998), whereas in this study birth order was significant for latency from birth to standing and Slee & Springbett (1986) reported that birth order did exert an influence on the time it took lambs to reach the udder.

Maternal cooperation score has been shown to improve significantly as the age of dam increases (O'Connor *et al.,* 1985, Lambe *et al.,* 2001, Brown *et al,* 2016) which is in accordance with the findings of this study. Merino ewes born as twins had a significantly higher maternal cooperation score (Brown *et al.,* 2016), whereas in this study birth order had no significant effect on the maternal cooperation score. Even though in this study birth type or order not was significant several studies in literature report that an increase in litter size has also been shown to increase maternal cooperation score (O'Connor *et al.,* 1985; Lambe *et al.,* 2001; Everett-Hincks *et al.*, 2005). H line lambs had dams with better maternal cooperation scores that should probably have allowed them to progress quicker to suckling.

3.5.3 Random effects

In Merino lambs estimates of h^2 for birth weight ranged from 0.18 to 023 (Mortimer & Atkins, 1995, Cloete *et al.,* 2001; Dixit *et al.,* 2001; Duguma *et al.,* 2002). The estimates of 0.15 for h^2 and 0.37 for m^2 accords well with values in literature, it is also consistent with the estimates obtained from other breeds (Neser *et al.,* 2001; Duguma *et al.,* 2002; Matika *et al.,* 2003; Safari & Fogarty, 2003; Van Wyk *et al.,* 2003, Hanford *et al.,* 2006; Brien *et al.*, 2010; Hatcher *et al.,* 2010). Safari *et al.* (2005) reported h^2 estimates of 0.21 in wool breeds, 0.19 in dual-purpose breeds, and 0.15 for meat breeds, whereas similar values for direct and maternal heritability were estimated for a flock consisting of multiple breeds and composites in a study be Everett-Hicks *et al.* (2014) (0.13-0.14 and 0.18-0.32 respectively). A direct heritability estimate of 0.32 were estimated in Malpura sheep, where the addition of covariance produced a high and negative estimate of r_{am} (Gowane *et al.*, 2010). In the same study the additive genetic heritability estimate for birth weight was 0.19.

Estimates of h^2, m^2, and r_{am} for length of parturition in lambs amounted to 0.06, 0.18, and -0.78 respectively. In Dormer and SA Mutton Merino lams h^2 was estimates at 0.04 and 0.03 and for m^2 estimates at 0.14 and 0.15 (Cloete *et al*., 2002c). These results are consistent with those obtained in the present study. Lambing ease has been reported to have a low heritability (0.09) (Brien *et al.,* 2010) and a direct heritability of 0.06 (Brown, 2007). The assistance required during birth was reported as being moderate (0.26) (Matheson *et al.,* 2012). It was also suggested that the heritability of deaths due to dystocia was higher than for other sources of death in US lambs when paternal halfsib procedures were applied (Smith, 1977).

Lamb survival had a h^2 estimate of 0.07 with c^2 also being 0.07, where the h^2 is similar to the direct heritability estimates in literature (Safari & Fogarty, 2003; Safari *et al.,* 2005; Olivier *et al*., 2009; Hatcher *et al.,* 2010). Cloete *et al.* (2009) reported that the values for heritability estimates in lamb survival increased with the age of the lamb, having a value of 0.28 overall. Heritability estimates for lamb survival

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to 3 days of age and survival to weaning had low estimates of heritability, of 0.014 and 0.010 respectively (Brien *et al.,* 2010).

For the latency from standing to apparently suckling, h^2 was estimated at 0.12 with a corresponding m^2 estimate of 0.11. Suckling assistance was found to have a moderate heritability (0.32) (Matheson *et al.,* 2012). In SA Mutton Merinos, h^2 of the latency between standing and apparent suckling was estimated at 0.08 with a c^2 estimate of 0.17 (Cloete *et al*., 2002c). In Brien *et al.* (2010) they reported a low estimated heritability value of 0.15 for the time taken to contact the udder.

Lambe *et al.* (2001) reported that the h^2 of maternal cooperation score is 0.13, which is higher than what is reported in this study, however the c^2 estimate of 0.19, with a repeatability estimate of 0.32. The authors concluded that including maternal cooperation score can be considered when aiming for genetic improvement. Genetic variation for maternal cooperation score is also found to be low (Everett-Hincks *et al.,* 2005). Phenotypic variance for Maternal Bond Score was 0.46, where the repeatability for the trait was high (0.49) with moderate direct (0.20) and maternal (0.23) heritability (Hatcher *et al.*, 2010).

3.5.4 Covariances

Lambs that were heavier at birth were likely to have longer parturitions. This relationship was particularly strong at the maternal genetic level (0.89). Comparable figures in the literature were 0.71 for Dormers and 0.35 for SA Mutton Merinos. Correlations between MBS and various bodyweight were negative but favourable (Brown *et al.,* 2016). On a phenotypic level, litter weight has previously been shown to be associated with length of parturition (Fahmy *et al*., 1997; Cloete *et al*., 1998a). Hatcher *et al.* (2010) reported the covariance between direct and maternal genetic effect for birth weight as -0.03. When birthing assistance was assessed along with lamb vigour and suckling assistance, Matheson *et al.* (2012) reported that genetic (0.54 to 0.80) and phenotypic (0.29 to 0.60) correlations of the traits were moderate as well as positive. Lamb birth weight had a positive and significant effect only on the time it takes the lamb to reach the udder (Dwyer *et al.*, 2005). In Suffolk lambs the weight at birth became an increasingly important factor in determining the latency to suck, where in the Scottish Blackface lambs in the same study birth weight had a significant effect on early righting behaviours and the time it took the lamb to suck, where heavier lambs were quicker than lighter lambs (Dwyer, 2003). Dwyer & Bünger (2012) showed that heavier lambs required less assistance to suck than smaller lambs. In a study by Arnold & Morgan (1975) the only significant correlation was between the length of parturition and the birth of single born Merino ewes.

Lamb survival to 3 days of age and weaning were strongly correlated at phenotypic and genotypic correlation, with negligible phenotypic correlations with birth weight. A low and negative genetic correlation (-0.32) was estimated between birth weight and lamb survival to 3 days of age (Brien *et al.,* 2010).

Corresponding correlations that were estimated in the study of Cloete *et al*. (2002c) were variable and inconsistent between breeds, and do not support or refute the correlations obtained in this study. At this stage, these trends can therefore not be considered as robust and generally applicable to sheep breeding ventures. Lambe *et al.* (2001) reported high genetic correlations between maternal cooperation score in different parities, which is accordance with this study. However, large standard errors were

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reported making it unreliable. Dwyer & Lawrence (1999a) found that a difficult birth had a significant effect on the activity of the lamb. In a study by Dwyer *et al.* (2005) the length of parturition had no significant effect on the behavioural latencies of the lamb.

When assessed as a trait of the lamb, virtually no genetic covariation was detected between the latency from standing to apparently suckling and maternal cooperation score. This relationship was, however, particularly strong on the maternal permanent environmental level. This result is a further indication of the important role dams play as far as the facilitation of successful suckling by their offspring is concerned (Cloete & Scholtz, 1998).

The age of the ewe had a significant effect on all neonatal lamb behaviours as an increase in the experience in the ewe was associated with a decrease in the latency to stand, udder-seeking and suckling behaviours and to perform righting behaviour as a neonate (Dwyer, 2003). Similar results were seen in a study by Dwyer *et al.* (2005) where lambs from first parity ewes were slower in all behaviours, except in the latency to suckle, than more experienced mothers.

3.6 Conclusions

The results in this study show that selecting ewes for multiple rearing ability did not significantly affect behaviours shown after parturition by the lambs as H line lambs did not differ significantly from L line lambs, which is not similar to results of other studies on the same resource population. A significant effect however has been birth type or order which has been shown to affect the survival of the lamb. Birth weight affected the length of parturitions as heavier lambs had longer parturitions but has also been shown to be linked to improved lamb vigour. The survival of lambs, particularly the survival of multiples, were improved in the H line from 1993 to 1997 (Cloete & Scholtz, 1998) and from 1998 to 2001 (Cloete *et al.,* 2002b).

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Chapter 4
Genetic and environmental (co)variance components and ratios for number of lambs weaned per ewe mated and perinatal behaviour of Merino ewes

4.1 Abstract

Data for lambing behaviour were recorded during 1993 to 2002 in a resource population that has been divergently selected from the same base population since 1986. Selection was either for or against number of lambs weaned per ewe joined (NLW), resulting in a High (H) line selected in an upwards direction and a Low (L) line selected in the downward direction. Data were available for the latency of stay on the birth site for 1003 ewe-year records to 2024 ewe-year records for NLW. Overall, H line ewes were superior to L line ewes for NLW. They also had shorter parturitions and cooperated better with the first suckling attempts of their offspring. Direct heritability estimates (h^2), assessed as trait of the ewe, were 0.04 for NLW, 0.17 for length of parturition, 0.07 for maternal cooperation score and 0.20 for the interval ewes remained on or near the birth site. Genetic correlations indicated that ewes with a high NLW would also have shorter parturitions. Maternal cooperation score was genetically related to the time spent on or near the birth site. The differences between the lines in terms of behaviour, generally favouring the H line, would be beneficial to lamb survival thus enhancing NLW. Genetic trends suggested divergence between the lines for breeding values for length of parturition, suggesting that parturitions became shorter in the H line and longer in the L line. The results and parameter estimates obtained, suggested that selection for improved NLW would yield desirable outcomes for ewe behavioural traits. Selection would putatively benefit animal welfare by reducing birth stress in ewes and lambs and by promoting maternal cooperation with first suckling attempts in ewes.

4.2 Introduction

Lamb mortality has been found to be a major constraint to efficient sheep production (Haughey, 1991; Brien *et al*., 2014). Supervision and confinement of breeding ewes to indoor pens during the perinatal period in the pastoral sheep production areas of the world, is the major contributing factors to this intensification. To improve the survival of lambs and ewe rearing ability under paddock conditions, selective breeding has been suggested (Le Neindre & Poindron, 1990; Lindsay *et al.,* 1990; Haughey, 1991; Brien *et al*., 2014). Selection for an improved NLW has previously resulted in improved maternal and lamb behaviour that favours the survival of the lamb (Cloete *et al.*, 2005a; Brien *et al*., 2014). Behavioural adaptations were contended to contribute to the selection responses in lamb survival in the pastoral areas (Alexander, 1988). Maternal behaviour attributes that contributes to the survival of the lamb is grooming, low-pitched bleats, showing no aggression towards the lamb, not deserting the lamb, co-operating with the lambs suckling attempts, recognising the lamb, and maintaining a close contact with the lamb (Cloete & Scholtz, 1998; Dwyer & Lawrence, 2005). Estimates of genetic (co)variances for peri-parturient behaviour in sheep are not widely published (Hinch, 1997). In some traits (e.g. length of

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parturition and neonatal progress of lambs (Alexander *et al.,* 1990; Cloete & Scholtz, 1998; Kuchel & Lindsay, 1999), breed and line differences may be indicative of genetic variation. Low to moderate direct and maternal heritability estimates were also recorded for such traits when estimated as traits of Dormer and SA Mutton Merino lambs (Cloete *et al*., 2002c). However, as new traits are analysed, it is common that genetic correlations are associated with large standard errors and that there is inconsistency between breeds, therefore, no robust conclusion can be found that is applicable to the industry in this respect.

This study reports results pertaining to environmental factors associated with peri-parturient behaviour of sheep as well as the genetic (co)variances among traits. The resource population was divergently selected for maternal multiple rearing ability since 1986 (Cloete & Scholtz, 1998). In contrast to the earlier study by Cloete *et al.* (2002c), this study focused on assessing perinatal behavioural traits as ewe traits, while also determining genetic correlations of these traits with NLW.

4.3 Materials and methods

4.3.1 Animals and location

Using maternal ranking values for lambs reared per joining, two lines of Merino sheep were divergently selected from the same base population since 1986. The details for the selection procedure of replacements can be found in the literature (Cloete *et al.* 2004b; 2009) while it is also detailed in Chapters 2 and 3. Replacements for the High (H) line were ewe and ram progeny of ewes that reared more than 1 lamb per joining (i.e. reared twins at least once). The Low (L) line replacements were from ewes that reared less than 1 lamb per joining (i.e. barren or lost all lambs, at least once).

Apart from during joining in single sire groups to 4-5 rams, the 2 lines were maintained as single flock since its establishment. Irrigated kikuyu (*Pennisetium clandestinum)* paddocks were used during joining in the summer (January – February) and during lambing in the winter (June – July) for the duration of this experiment. There were 10 kikuyu paddocks on which lambing took place of approximately 0.3 to 0.5 ha each, as described in detail by Cloete *et al.* (2002b) For the remainder of the year, dryland lucerne *(Medicago sativa)* and medic *(M. truncatula)* pastures were used. Occasionally during winter, an oat *(Avena sativa)* fodder crop was used, whilst oat crop residues and standing hay were sometimes available during spring and early summer. Supplementary feeding was rarely needed as nutrition was mostly adequate throughout the year. Ewes were annually shorn within 3-4 weeks before the lambing commenced. The study was conducted over a 10-year period from 1993 to 2002.

4.3.2 Observations and recordings

During peak lambing (approximately 3 weeks) the lambing flocks were continuously observed by 1 to 2 trained observers. The ewes quickly adapted to the presence of the observers and could quite soon be approached closely (< 10m) without unnecessary disturbance. Five lambing paddocks were reserved for usage during daytime while another five were floodlit for usage at night (Cloete & Scholtz, 1998). The behaviour traits that were recorded included the length of parturition of individual lambs, which was defined as the latency between the first definite sign of parturition and the birth of the last lamb. Assistance was provided to ewes failing to deliver within 3.5 hours from the commencement of parturition (Cloete *et al.,*

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2002a). However, a few ewes were assisted if they had not delivered 2 hours after the any part of the lamb was presented at the vulva or 30 minutes after the obvious malpresentation of body parts (Cloete & Scholtz, 1998). The time prior to assistance was accepted as the length of parturition as an ewe trait.

The birth site was marked with a peg and the movement of the ewes from the birth site was recorded. If ewes moved away from the birth site for more than 2 hours and covered a distance exceeding 15 m, it was regarded that the ewe permanently left the site. A grid system consisting of iron fence posts and fluorescent markers were used to aid in the estimation of distances. The posts and markers were placed at 20 m intervals on paddock fences (Cloete & Scholtz, 1998). Behaviour that were recorded during suckling attempts, were those that frustrated the first attempts of the neonate, including backing, circling, and/or butting (Alexander, 1988). The behaviours that aided in the neonates first suckling attempts were also recorded. These included standing still, adopting a slightly hunched posture to enable access to udder, and nudging the lamb in a position that will facilitate suckling. These recordings were accumulated to give the ewe and overall maternal cooperation score on a 6-point scale, where a positive score was given for each of the distinct behaviour that aided in the neonates suckling attempts, and similarly a negative score was given for each distinct behaviour that did not aid in the neonates suckling attempts. The scores allocated for the assistance with the first suckling attempts of the individual lambs were averaged for parities of individual ewes in litters exceeding one lamb (Cloete & Scholtz, 1998). NLW were recorded as described in detail by Cloete et al. (2004a).

4.3.3 Statistical analysis

The latency traits (length of parturition and stay on the birth site) did not meet the requirements for analysis by linear methods as far as skewness and kurtosis were regarded. These traits were thus transformed to natural logarithms after 3 were added to account for very low latencies. The ASREML program (Gilmour *et al*. 1999) was used for the estimation of the fixed effects, and to subsequently to derive variance for NLW as well as behavioural traits in univariate analyses. Fixed effects fitted for the behavioural traits were year of lambing (1993-2002), selection line (H and L), the number of lambs born (single or multiple) as well as dam age (2-7+ years). The number of lambs born was not fitted for NLW. The first analyses involved fitting various combinations of fixed effects and interactions between them to obtain an operational model. Significant ($P<0.05$) effects in these preliminary analyses were retained in subsequent analyses. Random terms were then added to the operational model, resulting in the following genetic models for analyses of ewe traits (in matrix notation):

$$y = Xb + Z_1a + e \quad (1)$$

$$y = Xb + Z_1a + Z_2c_{PE} + e \quad (2)$$

$$y = Xb + Z_1a + Z_3c_{ssire} + e \quad (3)$$

$$y = Xb + Z_1a + Z_2c_{PE} + Z_3c_{ssire} + e \quad (4)$$

In these models, y was a vector of observations for NLW and ewe behavioural traits, b, a, c_{PE} and c_{ssire} were vectors of fixed effects, direct genetic variances, ewe permanent environmental variances and

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random service sire permanent environmental variances respectively, X, Z_1, Z_2, and Z_3 were the corresponding incidence matrices relating the respective effects to y, and e was the vector of residuals. All analyses included the full pedigree file, consisting of 4351 individuals, the progeny of 216 sires and 1107 dams.

It was assumed that:

$V(a) = A\sigma^2_a$; $V(m) = A\sigma^2_m$; $V(c) = I\sigma^2_c$; $V(e) = I\sigma^2_e$,

With I being an identity matrix; σ^2_a, σ^2_{PE}, σ^2_{SSIRE} and σ^2_e being the direct genetic variance, maternal genetic variance (as a trait of the ewe), the ewe permanent environmental variance, the service sire permanent environmental variance and the environmental (residual) variance respectively.

Log likelihood tests were conducted to determine the most suitable model for each trait in single-trait analyses. The random effects models that best fitted the data for each trait were identified, using the log likelihood ratios obtained from the respective analyses (Snyman *et al*., 1995). Subsequently, a four-trait animal model was fitted, including all traits. These analyses allowed the calculation of all relevant direct and maternal correlations between traits, together with their appropriate standard errors.

Direct breeding values for traits were obtained for ewes and regressed on lambing years using standard regression techniques. The intercept was set at zero for the 1986 animals, as they were the first unselected progeny group. Genetic trends were inspected for divergence between the lines, using standard errors obtained for the regression coefficients. Breeding values used for this were obtained from analyses where selection line and its interactions with other traits were excluded from the operational model. Including selection line as fixed would reduce genetic differences between lines that accrued as a result of selection.

4.4 Results

4.4.1 Descriptive statistics

The descriptive statistics for traits are given in Table 4.1. Coefficients of variation for the behavioural traits ranged from 15.6% for the time ewes remained on or close to their birth sites to 21.8% for the length of parturition (Table 4.1). The time ewes remained on or close to their birth sites remained slightly leptokurtic even after transformation.

Table 4.1 Descriptive statistics for the traits considered in the study

Parameters	Trait			
	Number of lambs weaned per ewe joined	Length of parturition (minutes)	Cooperation with first suckling attempts	Stay on birth site (minutes)
Number of observations	2024	1117	1063	1003
Mean±SD	0.94±0.69	3.95±0.86	5.21±1.06	5.69±0.89
Range	0-3	1.39-6.82	1-6	1.39-7.27
Skewness	0.12	0.04	-1.50	-1.27
Kurtosis	-0.74	-0.34	1.80	3.09

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4.4.2 Fixed effects

Selection line affected all traits with the exception of the time ewes remained on or close to their birth sites ($p<0.05$; Table 4.2). Year effects were mostly significant, except for length of parturition. Ewe age affected all traits while the number of lambs born was also significant where it was fitted. The only interaction reaching significance ($p<0.05$) was that of selection line x ewe age for length of parturition.

Table 4.2 Significance of fixed effects and interactions with an effect on the traits observed

Fixed and interaction effects	Trait			
	Number of lambs weaned per ewe joined	Length of parturition (minutes)	Cooperation with first suckling attempts	Stay on birth site (minutes)
Selection line (SL)	**	*	**	0.183
Year	**	0.097	**	**
SL x Year	0.799	0.158	0.858	0.922
Age	**	*	**	*
SL x Age	0.506	*	0.781	0.949
Lambs born (LB)	-	**	**	**
SL x LB	-	0.871	0.743	0.664

* Significant ($p<0.05$); ** Significant ($p<0.01$); Actual significance for $p>0.05$; - No significance

Relative to L line ewes, their contemporaries in the H line had a 47% advantage for NLW ($p<0.01$; Table 4.3). NLW increased ($p<0.05$) from 2-year-old ewes to peak at 4 years, before declining again to the level of 2-year-old ewes for the 7+ year-old-ewe group. Overall, H line ewes had shorter parturitions than their L line contemporaries ($p<0.05$).

It was evident that the parturitions of H line ewes were generally shorter than those of their L line contemporaries, significantly so at 2, 3 and 5 years of age. This pattern was disrupted in 6-year-old ewes, with the line for L line ewes dipping slightly below that of H line ewes at this age. Single-bearing ewes had shorter parturitions than their contemporaries with multiples. No clear pattern emerged for length of parturition and relation to ewe age. Maternal cooperation with first suckling was significantly affected by selection line, number of lambs born and the age of the ewe ($p<0.01$). H line ewes had a higher maternal cooperation score than L line ewes ($p<0.01$), and it improved with the age of the dam up until 4 years after which little improvement was seen. The time the ewe spent on the birth site was independent of the selection line. Ewes that cared for multiple lambs stayed on the birth site for longer periods of time than ewes that had single. The time spent on the birth site also increased as ewes aged ($p<0.05$).

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Table 4.3 Length of parturition (as a trait of the ewe), maternal cooperation score and the interval that ewes remained on or within 15 m from their birth sites (± s.e.). The latency indicating time intervals were analysed after transformation to natural logarithms after 3 was added to account for low values. Back transformed means are given in parentheses.

Fixed effects	Number of lambs weaned per ewe joined	Length of parturition (minutes)	Cooperation with first suckling attempts	Stay on birth site (minutes)
Number of observations	2024	1117	1063	1003
Overall mean	0.86±0.03	4.04±0.06 (53.8)	5.20±0.06	5.67±0.06 (288)
Birth year	**	0.10	*	**
Selection line	**	*	**	0.18
H line	1.03±0.03	3.95±0.07 (47.2)	5.40±0.07	5.77±0.08 (318)
L Line	0.70±0.04	4.16±0.09 (61.4)	5.00±0.09	5.58±0.09 (261)
Number of lambs born	N.A.	**	*	**
Single	-	3.83±0.06 (43.2)	5.30±0.06	5.59±0.07 (265)
Multiple	-	4.25±0.07 (66.8)	5.09±0.07	5.75±0.07 (313)
Ewe age (years)	**	*	**	**
2	0.70±0.04	4.10±0.09 (63.4)	4.39±0.09	5.52±0.09 (245)
3	0.88±0.04	4.02±0.08 (53.8)	5.10±0.08	5.62±0.08 (273)
4	1.01±0.04	3.96±0.08 (49.4)	5.33±0.08	5.55±0.08 (255)
5	0.97±0.04	4.05±0.08 (54.4)	5.41±0.08	5.76±0.08 (313)
6	0.87±0.04	3.89±0.08 (45.9)	5.46±0.09	5.70±0.09 (295)
7	0.76±0.07	4.11±0.14 (57.7)	5.50±0.17	5.89±0.17 (360)

* Significant (p<0.05); ** Significant (p<0.01); Actual significance for p>0.05; - No significance

4.4.3 Random effects and (co)variance ratios

Log likelihood ratios for the behavioural traits assessed in ewes are presented in Table 4.4. The inclusion of direct additive animal variances improved (p<0.05) the log likelihood ratios in all cases, when compared the fixed effects only model. The likelihood ratio test indicated that the addition of ewe permanent environmental variances improved the fit of the model for NLW and the maternal cooperation score with the first sucking attempts of the lamb(s). The log likelihood ratio for the length of parturition was further improved when random permanent environmental effects of the service sire were added to the model of analysis.

Table 4.4 Log likelihood ratios for the behavioural traits in Merino ewes of the H and L lines under four different models of analysis. The "best' models are denoted in bold, italic figures.

Model fitted	Number of lambs weaned per ewe joined	Length of parturition (minutes)	Cooperation with first suckling attempts	Stay on birth site (minutes)
Fixed effects only	-216.905	-388.343	-566.122	-410.178
+ h^2	-186.963	-370.401	-552.776	***-391.972***
+ h^2 + c^2_{PE}	***-179.091***	-369.061	***-550.170***	-390.933
+ h^2 + c^2_{ram}	-185.154	***-367.621***	-552.738	-391.972
+ h^2 + c^2_{PE} + c^2_{ram}	-177.652	*-365.949*	-550.152	-390.933

(Co) variance ratios: h^2 = direct additive; m^2 = maternal additive; c^2= maternal permanent environment; r_{am} = genetic correlation between direct and maternal effects

The direct heritability (h^2) estimates derived from single-trait analyses amounted to 0.04 for NLW, 0.18 for length of parturition, 0.07 for maternal cooperation, and 0.19 for the latency ewes remained on or

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near to their birth sites (Table 4.5). Expressed relative to the corresponding standard error (0.05), the h^2 for maternal cooperation score did not reach significance. The animal permanent environmental variance ratio reached significance for NLW (0.14) and maternal cooperation score (0.13). The permanent environmental variance attributed to service sires affected length of parturition. However, it only accounted for 3% of the observed phenotypic variance.

Table 4.5 Single-trait variance components as well as estimates of the direct genetic (h^2), animal permanent environment (PE – c^2_{pe}) and service sire permanent environmental (PE – c^2_{sire}) variance ratios (±s.e.) for the respective behavioural traits assessed in Merino ewes of the H and L lines

Variance components or ratios	Number of lambs weaned per ewe joined	Length of parturition (minutes)	Cooperation with first suckling attempts	Stay on birth site (minutes)
Variance components				
Direct additive (σ^2_a)	0.0175	0.1214	0.0695	0.1441
Animal PE (σ^2_{pe})	0.0581	–	0.1255	–
Service sire PE (σ^2_{sire})	–	0.0214	–	–
Residual (σ^2_e)	0.3513	0.5406	0.7859	0.6187
Total phenotype (σ^2_p)	0.4269	0.6634	0.9809	0.7628
Variance ratios				
h^2	0.04±0.03	0.18±0.04	0.07±0.05	0.19±0.04
c^2_{pe}	0.14±0.02	–	0.13±0.06	–
c^2_{sire}	–	0.03±0.02	–	–

– Effect not significant according to log likelihood ratio

Estimated h^2-values from the four-trait analysis were either very close or similar to those stemming from single-trait analyses (Table 4.6). The exception was maternal cooperation score where some between-ewe variation was repartitioned from the ewe permanent environment to additive genetic effects. The genetic correlation of NLW with length of parturition was favourable at -0.51, suggesting that ewes with shorter parturitions would perform better for NLW. The genetic correlation of NLW with maternal cooperation score was high but did not reach statistical significance. It was furthermore evident that ewes with higher maternal cooperation scores for facilitating suckling of their progeny also remained on their birth sites for extended periods (r_G=0.71). Phenotypic correlations were mostly similar in sign compared to genetic correlations, but smaller in magnitude. The four-trait ewe permanent environmental variance ratio was similar to the single-trait value for NLW, but smaller for maternal cooperation score. The animal permanent environmental correlation between these traits amounted to 0.24±0.22. The service sire permanent environmental variance ratio for length of parturition was 0.03 in the four-trait analysis, as it was in the single-trait analysis.

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Table 4.6 Four-trait variance component estimates (± s.e.) for the respective behavioural traits assessed in Merino ewes of the H and L lines

Variance components, ratios and traits	Trait			
	Number of lambs weaned per ewe joined (NLW)	Length of parturition (minutes) (LPART)	Cooperation with first suckling attempts (COOP)	Stay on birth site (minutes) (SOBS)
Variance components				
Direct additive (σ^2_a)	0.0197	0.1195	0.1002	0.1482
Animal PE (σ^2_{pe})	0.0572	–	0.0929	–
Service sire PE (σ^2_{sire})	–	0.0223	–	–
Residual (σ^2_e)	0.3506	0.5437	0.7904	0.6162
Total phenotype (σ^2_p)	0.4277	0.6855	0.9834	0.7643
(Co)variance ratios (h^2) in bold italics on the diagonal, genetic correlations below the diagonal and phenotypic correlations above the diagonal				
NLW	***0.05±0.03***	-0.11±0.03	0.02±0.04	0.02±0.04
LPART	-0.55±0.25	***0.17±0.04***	0.02±0.04	0.01±0.03
COOP	0.62±0.27	0.12±0.22	***0.10±0.05***	0.19±0.03
SOBS	0.10±0.25	0.21±0.17	0.69±0.20	***0.19±0.04***
(Co)variance ratios (pe^2) in bold italics on the diagonal, permanent environmental correlations below the diagonal				
NLW	***0.13±0.03***	–	–	–
COOP	0.26±0.27	–	***0.09±0.05***	
c^2_{sire}	–	0.03±0.02	–	–

– Effect not significant according to log likelihood ratio

4.4.4 Predicted breeding values and genetic trends

Behavioural records on lambs were obtained only for the period from 1993 to 2002, when the study on lambing and neonatal behaviour took place. Ewes born as early as 1986 were observed at an age of 7 years when they lambed during 1993, thus contributing data to the study. Since the first, unselected progeny group born in the selection experiment was born during 1986, predicted breeding values for ewe traits were obtained for all individuals born during the period from 1986 to 2002. These breeding values allowed for the construction of genetic trends right from the commencement of selection. Genetic trends were only reported for the behavioural traits, as genetic trends for NLW were reported previously.

Table 4.7 Regressions of individual breeding values on birth year from 1986 to 2002 for 2738 H and 1536 L line individuals. The intercepts of these regressions were set to zero as 1986 was seen as the commencement of the selection experiment.

Behavioural trait	H line		L line	
	Slope±s.e	R^2	Slope±s.e	R^2
Length of parturition (minutes)	-0.516±0.015**	0.30	0.524±0.023**	0.25
Cooperation with first suckling attempts	0.0071±0.0003**	0.17	-0.0014±0.0004**	0.43
Stay on birth site (minutes)	0.794±0.086**	0.03	-0.191±0.010**	0.18

**-P<0.01

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Regressions applicable to ewe behavioural traits were significant (p<0.05) in all cases (Table 4.7). The regressions suggested that births became longer in the L line and shorter in the H line with time (see Figure 4.2 as an example). H line ewes improved their cooperation with the first suckling attempts of their offspring and stayed increasingly longer on their birth sites as time went on. In contrast, the cooperation of L line ewes with the first suckling attempts of their lambs became poorer and they increasingly spend shorter times on or near to their birth sites.

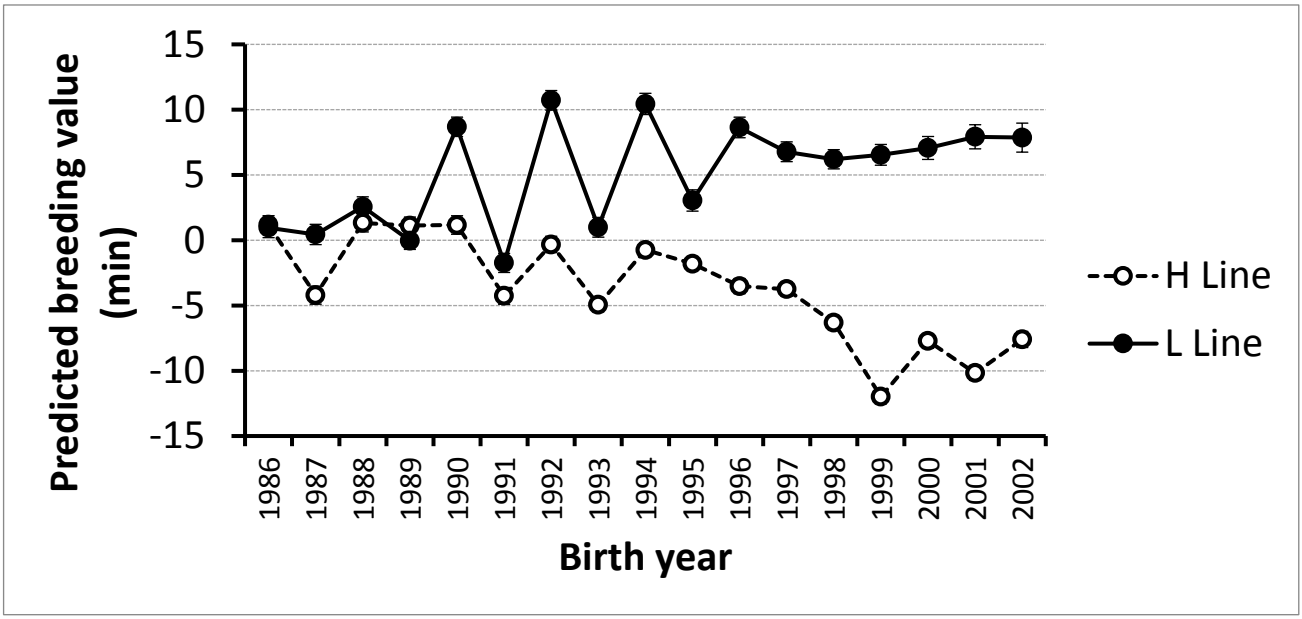

Figure 4.1 Birth year means (±SE) for back transformed breeding values for length of parturition of H and L line ewes describing the genetic trends in these lines.

4.5 Discussion

4.5.1 Descriptive statistics

Analyses were continued for the time ewes remained on or close to their birth sites, despite a slight deviation from normality as far as kurtosis was concerned. Such deviations are not considered to have a large impact on the outcome of analyses. The coefficient of variation of 73% for NLW was high, but consistent with previous values (Cloete *et al*., 2004a; Safari *et al*., 2005; Brown *et al*., 2016).

4.5.2 Fixed effects

The marked line difference for NLW was expected as it was also reported in previous publications involving the same resource flock (Cloete & Scholtz, 1998; Cloete *et al.,* 2004b; 2017). These results demonstrate the feasibility of improving NLW by purposeful selection. Previous results on length of parturition of ewes suggested shorter parturitions in H line ewes only when a correction for larger litter weight was applied to the data (Cloete & Scholtz, 1998). The latter authors used a subset of about half the data being available for this study. They also concluded that maiden L line ewes were more likely to be assisted at birth than their H line contemporaries. Previous results accordingly indicated that length of parturition depended on breed, also suggesting a genetic basis for this behavioural trait (Cloete, 1992). In

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Suffolk ewes bearing single lambs, the need for birthing assistance was significantly higher than in ewes bearing twins (Dwyer & Lawrence, 1998). This effect was, however, not seen in Blackface ewes where the assistance required did not differ between single and twin lambs. Moreover, breeds selected for terminal sire traits (Texel and Suffolk) in a study by Dwyer & Bünger (2012) showed an impaired ability to lamb without assistance. Their lambs also had lower lamb vigour scores than the other breeds used (Scottish Blackface and Mule x Texel). The authors concluded that it is possible that assisting ewes during birth may have reduced the natural selection for birthing ease and large pelvic dimensions in ewes. This line of reasoning supports the line difference reported here for length of parturition in the H and L lines. The interaction of selection line with ewe age appeared to mostly stem from a random, unexpected response in 6-year-old ewes (Figure 4.1) and not from a trend suggesting that it resulted from the underlying biology associated with selection for NLW. It should thus be interpreted with caution as it may have been quite a random coincidence.

The higher levels of maternal cooperation score by H line ewes with the first suckling attempts of their offspring could be linked back to results that maiden H line ewes were less likely to back away from the first suckling attempts of their lambs than L line contemporaries (Cloete & Scholtz, 1998). Mature H line ewes were also less likely to circle than their flock mates in the L line. Maiden H line ewes were more likely to aid the first suckling attempts of their lamb(s) by active cooperation by adopting a posture to facilitate suckling in the latter study while a similar tendency was observed in mature ewes ($p<0.10$). After this behaviour has been accounted for by analysis of covariance, a line difference in the latency of lambs to progress from standing to suckling still remained. This result led to the contention by Cloete & Scholtz (1998) that lambs may also have an innate ability to quickly progress to suckling.

The selection of the birth sites has been shown to an important factor in preventing the separation of ewe and lamb (Dwyer *et al.*, 2015) while ewes remaining on their birth sites for longer periods were less likely to desert their lambs compared to ewes leaving their birth sites soon after birth (Cloete, 1992). No conclusive line difference was found for the time ewes remained on or near their birth sites ($p<0.18$) although absolute differences favoured the H line. The previous study by Cloete & Scholtz (1998) also suggested no evident line difference in the period ewes remained on or near their birth sites. In this respect, Kilgour & Szantar-Coddington (1995) found that Fertility flock Merino ewes remained on their birth sites for 266 minutes compared to 251 minutes for control ewes ($p>0.05$). Knight *et al*. (1988) found that 41 Marshall Romney ewes grazed from their birth sites 46 minutes after birth, while 16 control Romneys did so after 27 minutes ($p>0.05$). A general pattern emerged that ewes selected for rearing ability or lamb survival tended to remain on their birth sites for longer periods than control ewes, although none of these studies found a significant line effect. Keeping in mind that this type of behaviour facilitates the formation of a strong mother-offspring bond (Alexander, 1988), as well as the heritable nature of the trait, this change in behaviour may well be a correlated response to selection. In the study of Murphy *et al*. (1994), it was shown that the presence of the ewe at the birth site as such did not improve the dam-offspring bond, since similar advantages could be obtained by penning ewes for 6 hours on another site. The authors also showed that the survival of multiple-born lambs is better when the ewe and the lamb remained in close

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contact for the first 6 hours after birth. The time that the ewe remained on the birth site was not significantly affected by age of the ewe and the number of lambs that was reared (Cloete, 1992). Merino ewes, compared to other breeds, have been shown to be poorer mothers and they tend spend less time at the birth site (Alexander *et al.*, 1983, 1990), which is similar to what was found in SA mutton Merinos, which remained at the birth site for a shorter length of time than Dormers (Cloete, 1992).

The improvement of NLW with an increase in age is consistent with previous results on Merino ewes (Cloete & Heydenrych, 1986; Cloete et al., 2003c; Kleeman & Walker, 2005). Length of parturition depended on dam age ($p<0.05$). However, no evident age trend could be determined except that 2-year-old ewes had longer parturitions than 6-year-old ewes (Table 4.3). Alexander (1960) also reported that 2-year-old ewes had longer parturitions than older ewes. Other studies did not find significant age the dam effects on length of parturition (Arnold & Morgan, 1975; Cloete *et al.,* 2002c; Dwyer, 2003).

Primiparous ewes have been shown to be less competent mothers and tend to experience longer parturitions (Dwyer, 2008). In several studies it has been reported that lambs born from 2-year-old ewes were shown to be less likely to survive than the lambs born from ewes that were older (Murphy *et al.*, 1994; Lopez-Villalobos & Garrick, 1999; Riggio *et al.,* 2008), whereas Everett-Hincks & Dodds (2008) reported that lambs born to 5-year old ewes had the lowest survival up until 3 days of age and lambs born from 2-year old ewes had the lowest survival up until weaning. Primiparous maiden ewes were more likely to engage in activities to discourage suckling (e.g. circling or backing) than experienced mothers (Alexander *et al*. 1993; Cloete & Scholtz 1998; Dwyer & Lawrence, 2000b).

The primary movement around the lamb is circling which was more prominent in primiparous than multiparous ewes, where primiparous ewes showed more backing movements which was non-existent in multiparous ewes (O'Connor *et al.*, 1992). A small percentage of 2-year-old ewes in a study by Alexander (1960) butted lambs vigorously during grooming and failed to stand still when the lamb was moving towards the udder. These behaviours were also reported by Dwyer & Lawrence (2002b) indicating that primiparous ewes were more likely to butt and withdraw from their lambs. The main responses ewes had to lambs' attempts at suckling was circling (Dwyer & Lawrence, 1999a) and walking away from the lamb (Dwyer & Lawrence, 2000a). Suffolk ewes, especially primiparous ewes, were more likely to show aggressive, rejection or fearful behaviour towards their lambs (Dwyer, 2008).

These activities would also be reflected in the maternal behaviour score used in this study. In a study involving 5-year-old primiparous single-bearing ewes, results of Alexander *et al*. (1993) indicated that there was no evidence of improved maternal attributes with an increase in ewe age that could not be related to previous experience of giving birth to and the rearing of lambs. It is contended that experience that accrued from previous reproduction opportunities in higher reproducing H line ewes could have contributed to the advantage of H line lambs cared for by younger ewes over their L line contemporaries. This argument does, however, not account for the better performance of inexperienced maidens in the H line. Everett-Hincks *et al.* (2005) described Maternal Behaviour Score (MBS) as an indication of the degree of the ewe's attachment to her lambs and/or the birth site vs. the ewe's attachment to the flock in the first 1 to 2 days following parturition when challenged by the presence of a human. Maternal behaviour score

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has been shown to improve significantly (P<0.01) as the age of ewe increases (Brown *et al.*, 2016). The latter study reported that ewes born as twins also had a significantly (P>0.05) higher maternal behaviour score in their combined and Merino datasets. Cloete & Heydenrych (1986) also reported that NLW was improved in twin ewes in comparison with singles.

O'Connor *et al.* (1985) reported that with an increase in MBS, the survival of the lambs also increases from birth to weaning. Latency from birth to standing was not influenced by the ewe not being able to stand still in a study by Arnold & Morgan (1975). Ewes caring for multiples had generally lower maternal cooperation scores than those caring for singles (Dwyer & Lawrence, 1998). This finding is probably related to distraction afforded by the birth process of siblings, or by the process of sharing attention between more than one offspring.

4.5.3 (Co)variance components and ratios as well as genetic trends

The low h^2 estimate derived for NLW is consistent with previous estimates of heritability for this trait (Cloete *et al.,* 2004a Safari *et al.,* 2007; Zishiri *et al.,* 2013; Bunter & Brown, 2015). Despite being lowly heritable, it is notable that marked genetic responses were reported previously for NLW (Cloete *et al.,* 2004; 2017). The previous studies, however, commanded greater animal resources and the h^2 of NLW could have been estimated with higher levels of precision. It is still reassuring the h^2 in present study compared favourably with those previous estimates. Duration of parturition was also heritable when analysed as a trait of the ewe (Tables 4.5 and 4.6). This result is consistent with breed and line differences in length of parturition (Cloete, 1992; Cloete & Scholtz, 1998; Dwyer & Bünger, 2012) as well as direct and maternal genetic components for length of parturition (Cloete *et al.,* 2002c; Chapter 3) and lambing ease score (Brown, 2007; Li & Brown, 2015). It is thus not surprising that selection for a correlated trait like NLW resulted in divergence in length of parturition as a correlated trait (Table 4.7 and Figure 4.2).

Maternal cooperation score had a small genetic component when assessed as a trait of the dam, although some between ewe variance repartitioned to animal additive effects in the four-trait analysis. It may well be that the line differences in the latency from standing to suckling in this study and in previous work on the same resource population (Cloete & Scholtz, 1998), could partly result from the divergent selection pressure for NLW in the lines on maternal cooperation score. This contention is supported by the divergence between lines in maternal cooperation score, assessed as a trait of the ewe. Hatcher *et al.* (2010) reported that maternal behaviour score was highly repeatable (0.49) and had a moderate direct (0.20) and maternal (0.23) h^2. However, the authors concluded that, for the specific flock under investigation, it was a poor indirect selection criterion for ewe rearing ability. In this study maternal cooperation were also heritable and was significant for all traits analysed, and of importance is with selection line. This indicated that selecting for multiple rearing ability would lead to the ewe having a better maternal cooperation score. Whether the indirect selection of maternal cooperation score for multiple rearing ability will have the same effect is still questionable and needs to be investigated further. When analysed as a trait of the ewe, the period that ewes remained on or near the birth site was also found to be heritable. We did not find any other evidence to support or refute the h^2 estimate in Tables 4.5 and 4.6,

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but it was supported by suggestions towards breed and line differences as reported under the previous heading.

The genetic correlation of length of parturition with NLW was significant and favourable, suggesting the ewes with shorter parturitions would perform better for NLW. The genetic correlation of NLW with dam cooperation score was quite high, although it did not reach significance. The genetic trends that are assumed to result as correlated responses to selection for NLW reported in Table 4.7 lend additional support to the existence of a sizable genetic correlation of NLW with maternal behaviour score. Clearly it would be beneficial to further pursue the genetic correlations listed above as no comparable results were found in the literature.

There was some evidence that maternal cooperation score was genetically related to the period that ewes remained on or near their birth sites. No directly comparable results could be found in the literature. A similar trait like MBS can only be useful as an indirect selection criterion if it is heritable as well while also being correlated genetically with survival (Brien *et al.,* 2014). Heritability estimates ranged from 0.09 to 0.20 (Lambe *et al.,* 2001; Everett-Hincks *et al.,* 2005; Hatcher *et al.,* 2010; Brown *et al.,* 2016). These results are similar to what is found in this study for maternal cooperation score. Repeatability ranged from low to high between 0.09 to 0.49 (Lambe *et al.,* 2001; Everett-Hincks *et al.,* 2005; Hatcher *et al.,* 2010).

Everett-Hincks *et al.* (2005) concluded that the low repeatabilities would indicate that a dam with a high MBS in one lambing year is unlikely to have similar MBS in the subsequent lambing years. Major sources of variation in MBS is environmental, with genetic variance being small (0.15) with considerable permanent environment effects (0.19) (Lambe *et al.,* 2001). The correlation between MBS and ewe parities were generally high with the phenotypic variance of MBS in each parity ranging from 1.20 to 1.31. It is well established that the birth site is of utmost importance in the formation of a strong dam-offspring bond (Murphy *et al*. 1994; Lindsay, 1996). Early neonatal lamb-ewe interactions are equally important in the formation of this bond (Lindsay *et al*. 1990; Murphy & Lindsay, 1996). When assessed from this perspective, such a relationship seems to be reasonable.

4.6 Conclusions

The line differences found in this study suggested that the H line was adapted to express behaviour characteristics that would enhance lamb survival. This was particularly true for those results pertaining to length of parturition and the maternal cooperation of the ewe with the lambs' first attempts at suckling. Increasing the experience of the ewe seemed to benefit the time spent by ewes at the birth site. The latter trait seemed not to be correlated with NLW although derive genetic trends suggested that the L line ewes spent less time at their birth sites as time progressed. Further research is needed to provide additional information on this as well as other issues that became apparent during the study.

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Chapter 5
Behavioural and production responses of H and L line lambs born as the progeny of random surrogate dams in an embryo transfer programme

5.1 Abstract

Mature 6- to 7-year old ewes subjected to divergent selection for number of lambs weaned per ewe joined (NLW) from the same base population from 1986 were used as embryo donors in an embryo transplant programme. Embryos harvested from these ewes were transplanted in random surrogate dams. Data for behavioural and production traits were recorded at lambing for 6-year period from 2009 to 2014. Birth weight, birth coat score, and weaning weight was independent of selection line, whereas it was significant for yearling lambs. Survival of H line lambs was higher than in L line lambs. H line lambs were also heavier at yearling age than L line lambs. H line lambs had substantially less wrinkles at the neck, body and breech as well as totalled across sites when compared to their L line contemporaries. Finer fibre diameters were observed in H line than in the L line. No line difference was found for FEWC in either yearlings or mature ewes. Embryo ewes used in this study were born in 2009 and lambed for the first time in 2011. H line breeding ewes from the embryo transfer programme had a higher reproduction than L line ewes. Lamb behaviours such as length of parturition, latency from birth to standing, latency from birth to suckling, latency form standing to suckling and maternal cooperation score was not significantly affected by the selection line or breed of the surrogate dam. Maternal cooperation score had a significant effect on the time for the lamb to stand after birth, where dam age was significant for maternal cooperation score. This indicates that the behaviour of the lamb may be affected by the experience of the ewe and may play a role in which behaviours the ewe exhibits.

5.2 Introduction

Multiple ovulation and embryo transfer (ET) in livestock are seen as a useful tool to increase selection intensity on the female side (Rathie, 1982), as it may increase the rate of genetic gain (Bari *et al.,* 2000; 2003; Granleese *et al.,* 2015). These genetic gains can be up to 50 to 100% faster than by conventional means in sheep and beef cattle (Smith, 1988). It is also useful to enhance sex limited traits with limited genetic variation (such as reproduction rate of sheep) by increasing the number of progeny produced by proven dams (Lang *et al.*, 1982).

Despite its obvious advantages with regard to the enhancement of genetic progress (Smith, 1988), industry acceptance of the concept of ET remains low (Bindon, 1988; Wuliji *et al.*, 1995). Results of applied ET programmes where its potential has been demonstrated is therefore scarce in the literature. It has been applied for the reduction of fibre diameter in a flock of ultrafine Merino sheep in New Zealand (Wuliji *et al.*, 1995). Progeny from the ET programme produced wool of 0.9 µm finer than that of contemporaries screened into the ultrafine flock. The difference relative to unselected control Merinos amounted to 2.5 µm. Resource flock ewes were similarly used to act as donor dams to propagate ewe numbers in a

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selection line that were selected for an increased number of lambs weaned per ewe joined (Cloete *et al.,* 1998). Although the number of progeny produced per donor ewe were low at 2.9, the reproductive performance of ET progeny were on par with those ewes that were selected in the upwards direction for 5-6 years, making the exercise successful in enhancing selection flock ewe numbers. The divergent selection experiment became one of the most important small stock resource flocks under the auspices of the Western Cape government (Schoeman *et al.,* 2010). These selection lines provided study material for numerous refereed papers, national and international presentations, and were instrumental in the postgraduate training of numerous students. However, downward selection is causing ewe numbers in the line selected in the downwards direction (the Low or L line) to dwindle rapidly. It is likely that this line will cease to exist unless drastic intervention is affected (Naidoo, 2012). Using ET to arrest the downward trend in L line numbers also opens up interesting research opportunities. Previous work has suggested that neonatal behaviour of ewes and lambs in the line selected in the upwards direction were adapted to enhance lamb survival (Cloete & Scholtz, 1998; Brien *et al.,* 2014). However, it was difficult to conclusively attribute line differences to either ewe or lamb factors. Lambs are given birth to and reared by random recipient ewes in an ET programme, making it possible to assess neonatal behaviour and survival as a trait of the lambs without being confounded by the genetic background of the dam.

Against this background and given the importance of meat production in South Africa (Cloete & Olivier, 2010), we conducted an ET programme with ewes screened on the basis of their prolificacy from South African medium wool Merino flocks as donors. The result of the increased selection intensity obtained from ET was evaluated against production levels in Merino lines divergently selected for ewe multiple rearing ability since 1986 (Cloete *et al.,* 2004a; 2009).

5.3 Material and Methods

5.3.1 Resource population

Mature 6- to 7-year-old ewes were selected out of two Merino lines that were divergently selected for number of lambs weaned per ewe joined (Cloete *et al.,* 2004a; 2009). The background of these lines was described by Schoeman *et al.* (2010) but a brief account of selection practices and the history of the flock will be provided. Divergent selection for number of lambs weaned commenced in 1986 and was initially based on a ranking table proposed by Turner (1978). After 2002, ranking values were augmented with repeatability model breeding values for the trait of interest (Cloete *et al.,* 2009). Selection was in a downward direction in the line selected for a low number of lambs weaned per joining (the Low or L line) and for high breeding values in the upwards selection line (the High or H line).

5.3.2 Preparation of donor and recipient ewes

The 6- to 7-year-old ewes acting as donors were selected on the maximum number of lambs weaned per parity in the H line and the minimum number of lambs weaned per parity in the L line. Recipient ewes were 18-month-old Merino and Dohne Merino ewes from the Langgewens flock (Cloete & Cloete, 2015),

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as well as 18-month-old Dormer ewes from the Elsenburg flock (Van Wyk *et al.,* 2003) and SA Mutton Merino ewes from the Elsenburg flock (Cloete *et al.,* 2004b).

The most widely used protocols for MOET consist of a pre-treatment with progestogen-impregnated intravaginal devices for 11-14 days, with FSH treatment beginning approximately 48-60 h before progesterone-releasing device removal (i.e., stimulating luteal and follicular phases). The FSH treatment consisted of 6-8 decreasing doses given twice daily.

Oestrus was synchronised in all donor ewes by the insertion progestogen-impregnated devices on day 0 of the programme and left *in situ* for a period of 12 days. Oestrus in recipient ewes were also synchronised in a similar way. When donor ewes were in oestrus, they were inseminated by intra-uterine insemination with fresh semen from respective rams and this was performed between 44-46 h after sponge removal. Embryos were collected from donor ewes on day 5 or day 6 after insemination (i.e. day 19 or 20 of the programme using a semi laparoscopic procedure under general anaesthesia. Embryos were stored in an ovum culture at room temperature since collection and remained there until the embryos are transferred laparoscopically as singles or twins.

5.3.3 Lambing management and records

At lambing in March 2009 to 2015, the ET progeny were identified with their recipient dams and linked back to their sire and donor dam of origin. Lambing took place in the paddocks prepared for this purpose (Cloete & Scholtz, 1998), where ewes grazed kikuyu (*Pennisetium clandestinum*). Neonatal ewe and lamb behaviours were recorded at lambing from 2010 to 2014 as described by Cloete & Scholtz, 1998). Lamb behaviour traits were length of parturition from the first indication of impending birth to the expulsion of the lamb, latency from birth to standing for more than 10 seconds, latency from birth to first suckling and latency from first standing to first suckling (see definitions of Cloete & Scholtz, 1998). The birth of a preceding twin was taken as the commencement of the birth of a subsequent litter mate. The reaction of the ewe to the first suckling attempts by the lamb was noted and combined as an aggregate score as described by Cloete *et al.* (2003c). Birth weight, recipient dam number and birth coat score (see Cloete et al., 2003b) were recorded within 24 hours of birth. Weaning weight was recorded at a mean (±SD) age of 111±7 days. Lamb losses were recorded between birth and weaning, allowing individual lamb survival records.

All surviving lambs were reared to yearling age and recorded for yearling live weight, greasy and clean fleece weight, clean yield, fibre diameter, the SD and coefficient of variation of fibre diameter, staple length and strength, subjective fold score (see Scholtz et al., 2010b) and testis dimensions in rams. Ewes selected as replacements entered the breeding flock at 18 months and were recorded for mating weight at joining in January-February and subsequent reproduction from 2011 to 2018. Lambing took place in June-July as described by Cloete *et al.* (2009). Records of ewes and lambs were combined to compile annual reproduction records for number of lambs born, number of lambs weaned and total corrected weight of lamb weaned as described by Cloete *et al.* (2004a). Faecal worm egg counts (FWEC) were additionally available for both yearling progeny as well as mature breeding ewes.

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5.3.4 Statistical analyses

Continuous data were analysed by least-squares methods since the data were typically unbalanced as is often the case with breeding data. ASREML (Gilmour *et al.,* 2015) was used for this purpose. The software allows the fitting of a range of random and fixed effects in mixed animal models. Random animal effects were fitted for the variance it controlled but were not reported in view of the small size of the data sets used. Latencies recorded for neonatal lamb survival were not normal distributed but were easily normalised by transformation to natural logarithms after 10 were added to account for very low values. Binomial lamb survival records were analysed by Chi-square methods (Van Ark, 1990). Ewe reproduction records were analysed according to the method proposed by Brown (1988).

5.4 Results and Discussion

5.4.1 Success of the programme

In total, 178 H line lambs (flushed from 29 donor ewes) were born from surrogate ewes, of which 163 were weaned. Corresponding values for the L line embryos flushed from 31 ewes were 172 and 130. Over the six years that the experiment ran, the average (±SD) number of lambs born and weaned per donor flushed amounted to respectively 6.1±1.1 and 5.6±1.1 for the H line. Corresponding values in the L Line were 5.5±3.3 and 4.1±2.7. When the coefficients of variation were considered, it was evident that the year to year variation in embryo yield per donor was substantially higher in the L Line compared to the H Line. However, the MOET programme was substantially more successful than the previous effort in the early 1990's. Cloete *et al.* (1998) reported a mean number of lambs born to be 2.9±2.5 per donor ewe. This figure included lambs borne from natural mating of donor ewes after the MOET programme was completed as well as those born from surrogate ewes. The donor ewes not cast for age were returned to the flock in the present study, to lamb in the winter of the year following their participation in the MOET programme.

5.4.2 Early growth and survival

Birth weight, birth coat score and weaning weight of the H and L line progeny produced by surrogate ewes were independent of selection line (P>0.05; Table 5.1). The results for birth weight and birth coat score were as expected (Cloete *et al.,* 2003b). In Chapter 3, selection line had a significant effect on the birth weight of the lambs, as the H line lambs were heavier than their L line contemporaries, which was not seen in MOET progeny. However, based on the results of the latter authors, a difference in weaning weight in favour of the H Line was expected. However, it should be noted that the absolute trend was in the expected direction (P=0.15), while only 163 H and 130 L Line lambs were available. It is feasible that significance could have been demonstrated if more lambs were produced. The survival of H Line lambs was improved relative to their L Line contemporaries based on a Chi-square test. This result is in accordance with Chapter 3 results, where the selection had a significant effect on the survival of the lambs, and the H line lambs had a better survival than their L line contemporaries.This result was expected based on previously published work (Cloete & Scholtz, 1998; Cloete *et al.,* 2009).

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Table 5.1 Least squares means (±s.e.) depicting means for the performance of H and L Line lambs reared by surrogate ewes for the traits recorded between birth and weaning

Trait	Selection Line		Significance
	H Line	L Line	
Birth weight (kg)	3.9±0.2	4.1±0.2	0.53
Birth coat score (n)	3.1±0.3	3.6±0.3	0.46
Weaning weight (kg)	23.7±1.6	20.2±1.8	0.15
Survival	0.916	0.756	**

** - P<0.01; Actual significance for P>0.05

5.4.3 Yearling performance

Production traits at yearling age of the progeny born in the H and L Lines from surrogate dams are reported in Table 5.2. H Line lambs were heavier than L Line contemporaries at yearling age with substantially less wrinkles at the neck, body and breech as well as overall. These results were expected based on previous work by Cloete *et al.* (2005a) and Van Wyk *et al.* (2014). Most wool traits were independent of selection line, with the exception as fibre diameter, where a tendency towards finer fibres was observed in the H line (P=0.08). This result was unexpected, based on previous results from the flock. A similar tendency was observed for scrotal circumference in favour of H Line yearlings (see Cloete *et al.*, 2005). No line difference was expected for FWEC, based on previous research by Mpetile *et al.* (2015).

Table 5.2 Depicting the means (± s.e) for the performance of H and L Line lambs reared by surrogate ewes for the traits recorded at yearling age

Trait	Selection Line		Significance
	H Line	L Line	
Yearling weight (kg)	42.3±2.5	34.7±2.8	*
Clean fleece weight (kg)	2.7±0.2	2.8±0.2	0.90
Clean yield (n)	3.1±0.3	3.6±0.3	0.60
Clean fleece weight (kg)	2.0±0.1	2.1±0.2	0.78
Staple length (mm)	57.2±2.0	58.8±2.1	0.59
Staple strength (N/ktex)	44.1±2.9	49.0±3.2	0.27
CV of fibre diameter (%)	17.7±1.0	18.9±1.1	0.41
Fibre diameter (µm)	18.3±0.4	19.3±0.5	0.08
Neck fold score (n)	2.5±0.2	4.0±0.3	**
Body fold score (n)	1.6±0.2	2.7±0.2	*
Breech fold score (n)	2.1±0.3	3.4±0.4	*
Total fold score (n)	6.2±1.6	10.1±0.8	**
Scrotal circumference (cm)	31.6±1.2	28.2±1.4	0.08
Testis diameter (mm)	98.0±3.1	86.6±3.5	0.23
Log of FWEC (+100)	6.5±0.3 (562)	6.6±0.4 (652)	0.81

* - P<0.05; ** - P<0.01; Actual significance for P>0.05

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5.4.4 Mature ewe production

The reproduction results found for ewes borne from surrogate ewes as part of the MOET programme are given in Table 5.3. Embryo ewes born in 2009 lambed for the first time in 2011. Therefore 253 lambing records from 2011 to 2015 were considered. The data represented a disproportionate percentage of records from young ewes, with 38.0% of records from 2-year-olds, 30.6% from 3-year-olds, 21.9% from 4-year-olds, 7.1% from 5-year-olds and 2.4% from 6-year-olds. Ewe mating weight was unaffected by selection line, although the absolute difference favoured H Line ewes (P=0.13).

Table 5.3 Least squares means (±s.e.) depicting means for the performance of H and L Line lambs reared by surrogate ewes for the traits recorded at yearling age

Trait	Selection Line		Significance
	H Line	L Line	
Mating weight (kg)	45.4±2.2	40.0±2.6	0.13
Number of lambs born	1.19	0.88	11.2**
Number of lambs weaned	0.91	0.67	8.4**
Log of FWEC (+100)	5.8±0.2 (232)	5.9±0.2 (252)	0.81

* - P<0.05; ** - P<0.01; Actual significance for P>0.05

Reproduction were higher in H Line ewes compared to L Line contemporaries when the Chi-square test adapted by Brown (1988) was used (Table 5.3). The log of FWEC was independent of selection line. Cloete *et al.* (2003a) reported that H Line ewes were heavier than their L Line contemporaries at 2 years of age. However, this age difference was reduced as ewes grew older with no line difference recorded in ewes older than 3 years. The catching up in live weight by L Line ewes was ascribed to the increased demands of pregnancy and lactation on H Line ewes because of their higher reproduction. The better reproduction of H Line ewes relative to their L Line contemporaries was expected and corresponds with previous results of Cloete & Scholtz (1998) as well as Cloete *et al.* (2003a; 2004a). The absence of a line difference in the log of FWEC was also consistent with expectations, as was also experienced in yearling progeny of the respective line.

5.4.5 Neonatal lamb behaviour

Group (H or L line) and breed did not significantly affect length of parturition, the time taken to stand and suckle from birth, the time from standing to suckling, or the maternal cooperation score of the ewe (Table 5.4, Table 5.5). Arnold & Morgan (1975) similarly found that breed did not have an effect on the length of parturition. Breed played a role in length of parturition in a study by Dwyer & Lawrence (1998) where Scottish Blackface ewes had shorter parturition times than Suffolk ewes. Regardless of ewe breed, Blackface lambs had shorter length of parturition the Suffolk lambs (Dwyer & Lawrence, 1999b). A probable cause for there being no difference between breeds with length of parturition in this study is that all the breeds used in this study from part of the Merino genotype and may also be the reason why no significant differences were seen in any of the lamb behaviours recorded nor in the maternal cooperation

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of the ewes. Blackface lambs were significantly quicker to perform all righting and sucking behaviours than Suffolk lambs, regardless of the ewe breed. However, there was a significant effect of ewe breed on the time taken to stand by Suffolk lambs, with lambs born to Blackface ewes being slower than if born to Suffolk ewes (Dwyer & Lawrence, 1999). In Dwyer & Lawrence (2000a) lambs born to Suffolk ewes were quicker to both stand and suck than lambs born to Blackface ewes. The birth status of the lambs had a significant effect ($p < 0.05$) on the length of parturition and the time taken for the lamb to stand from birth. Twins had longer lengths of parturitions than singles (Owens *et al.,* 1985; Cloete, 1992). Dwyer *et al.* (2005) where twin lambs were generally quicker than both singles and triplets in their initial behaviours, thereafter triplets were significantly slower than singles or twins. Results from Slee & Springbett (1986) and Dwyer & Lawrence (1998) were not in accordance with this study in that progress to standing was independent of birth type in lambs from several breeds.

Table 5.4 Depicting means (± s.e.) for the performance of H and L Line lambs reared by surrogate ewes. Back transformed means of traits are given in parentheses.

Trait	Selection Line		Significance*
	H Line	L Line	
Number of records	130-133	113-120	
Length of parturition (min)	3.79 ± 0.09 (34.3)	3.80 ± 0.10 (34.8)	0.93
Birth to standing (min)	3.38 ± 0.07 (19.3)	3.45 ± 0.08 (21.5)	0.47
Birth to suckling (min)	3.92 ± 0.05 (40.9)	4.04 ± 0.06 (46.6)	0.13
Standing to suckling (min)	3.32 ± 0.06 (17.7)	3.47 ± 0.07 (22.3)	0.08
Maternal cooperation score	5.14 ± 0.12	5.21 ± 0.14	0.65

*Actual significance for P>0.05

Ewes were evaluated for their (maternal) cooperation during the lamb's attempts to suckle. The behaviours of note were standing and adopting a suckling posture, which were considered positive behaviours, whereas circling, backing away, and pushing the lamb were the negative behaviours. For the time taken to suckle from birth, maternal cooperation was significant ($p < 0.05$), which indicate that the behaviour the ewe expresses during the lambs attempts to suckle does play a role in how quickly the lamb succeeds. Dam age was significant ($p < 0.05$) for maternal cooperation which indicates that the experience of the ewe may play a role in which of these behaviours the ewe exhibit during the lamb's attempt to suckle. This accorded with other findings where the maternal cooperation score improves with dam age (O'Connor *et al.* 1985; Lambe *et al.,* 2001; Brown *et al.,* 2016).

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Table 5.5 Least squares means (±s.e.) length of parturition, birth to standing, birth to suckling, and standing to suckling. Back transformed means are given in parentheses.

Trait	Breed				Significance
	Merino	Dormer	Dohne	SAMM	
Number of records	80-84	94-97	22-23	47-49	
Length of parturition (min)	3.73 ± 0.10 (31.6)	3.82 ± 0.09 (35.7)	3.74 ± 0.19 (32.2)	3.89 ± 0.12 (38.9)	0.52
Birth to standing (min)	3.46 ± 0.08 (21.9)	3.33 ± 0.07 (18.1)	3.45 ± 0.13 (21.7)	3.40 ± 0.09 (20.2)	0.61
Birth to suckling (min)	3.94 ± 0.07 (41.3)	4.05 ± 0.06 (47.1)	4.03 ± 0.12 (46.5)	3.90 ± 0.08 (39.4)	0.39
Standing to suckling (min)	3.32 ± 0.08 (17.7)	3.54 ± 0.07 (24.4)	3.38 ± 0.14 (19.5)	3.35 ± 0.09 (18.4)	0.16
Maternal cooperation	4.94 ± 0.15	5.04 ± 0.13	5.65 ± 0.27	5.07 ± 0.18	0.13

*Actual significance for P>0.05

5.5 Conclusion

The MOET programme implemented in this project seemed to be successful, as reflected by the number of lambs born and reared relative to the donor ewes treated. The performance of progeny from the MOET programme was generally in correspondence with expectations, namely H Line lambs survived better than L Line contemporaries between birth and weaning, they grew out to heavier yearling weights and were smoother compared to L line yearlings. In correspondence with literature cited, H Line lambs tended to be heavier at weaning with a larger testis diameter. H Line ewes also outperformed L Line contemporaries for reproduction, in line with previous reports in the literature. Reproduction results should be allowed to accrue for a longer period to ensure that unbiased estimates of the reproduction of the two selection lines could be derived. MOET can thus be used to propagate scarce genetic resources, such as the L Line in this study.

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Chapter 6
Arena behaviour of Merino weaners is heritable and affected by divergent selection for lambs weaned per ewe mated

6.1 Abstract

Data of 2291 6- to 8-month-old Merino weaners from two divergently selected lines at Elsenburg were used to evaluate the genetics of the response of lambs to humans in an arena test. The High (H) line was selected upwards and the Low (L) line were selected downwards for number of lambs weaned per ewe mated (NLW) from 1986 to the present. Selection resulted in two widely divergent lines for NLW, live weight at all ages, wrinkle scores as well as other breech strike indicator traits such as breech and crutch cover scores. Animals from the H line (selected for reproduction) came closer to a human operator seated in the arena between the tested animal and its contemporaries. They were also less likely to urinate and defecate, while they travelled longer distances based on the number of squares crossed in the arena, than their L line contemporaries (all $P<0.05$). The distance the lambs maintained from the human operator (0.08±0.03), urinating events (0.13±0.03), and defecating events (0.04±0.02) were all lowly heritable. However, the number of lines crossed (0.22±0.04) and the number of bleats (0.35±0.05) were both moderately to highly heritable. Both the genetic (0.39±0.18) and the phenotypic (0.22±0.02) correlations suggested that the animals that maintained a larger distance from the human operator covered a greater distance in the arena. Genetic trends indicated that, over time, L line animals increased their distance from the human operator while they also urinated and defecated at a higher frequency. H line animals, in turn, exhibited fewer urination and defecation events over time. Selection for NLW in the H line therefore resulted in a range of behaviours that would indicate lower levels of stress when interacting with humans in the Arena. Animal welfare is facilitated by lower levels of stress as well as good animal-stockperson relations during unfamiliar procedures.

6.2 Introduction

According to Dodd *et al.* (2012), temperament is defined as the behavioural responses of an animal to a stimulus that induces stress. Such responses can be facilitated by genetic as well as permanent environmental effects inherent to animals. It may also be the behavioural expression of fearfulness when an animal is being handled or is in contact with a human (Brown *et al.*, 2016) Behavioural responses (indicative of temperament of the animal) can range from showing docility to expressing fear or nervousness, non-responsive behaviour, escape or withdrawal behaviour and aggressive behaviour (Burrow, 1997). The ease of handling in both open and more restricted situations are important to producers as temperament was in the past also linked to production traits of economic importance (Dodd *et al.*, 2012) such as growth of lambs and rearing ability of ewes (Murphy *et al.*, 1994; Kilgour & Szantar-Coddington, 1995). In cattle, poor temperament is perceived to influence profitability by increased production costs as well as decreased production that results from the correlations between temperament and production traits (Burrow, 1997).

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Pale, soft and exudative meat in pork and dark, firm and dry meat in beef are both syndromes that may be the result of either acute short-term or chronic long-term stress. Selecting against the number of lambs weaned per ewe mated (NLW) resulted in inferior meat quality expressed by meat pH profiles in a study by Cloete *et al.* (2005a). It was suggested by Murphy *et al.* (1994) that animals of quiet temperament grew faster and were better producers than animals with restless, nervous or aggressive temperaments. Pajor *et al.* (2008) found that calm lambs had a higher live weight, and better relative and absolute weight gains than nervous animals. Selecting for the temperament of an animal may be seen as a potential way to reduce the animal's response to stress without having to alter management (Dodd *et al.*, 2012). Direct selection for ewe rearing ability poses its own set of problems, as it can only be measured for the first time at first lambing in ewes while the trait is not expressed in rams. A possible indirect selection criterion that can solve these two problems is measuring the behaviour of the animals in an arena test. Advantages of a proposed arena test include: 1) it can be measured as early as 6 months of age; 2) the arena behaviour of rams can be measured; 3) potential useful arena behaviour measurements, such as the distance travelled and the number of bleats, are continuously distributed; 4) arena behaviour is much easier to record than the lambing and rearing performance of individual ewes (Kilgour, 1998). When the arena test was first developed by Fell & Shutt (1989) it was used to quantify the aversion of animals to human presence after surgical stress. The latter study initially tested four animals simultaneously whereas individual animals were tested in the current arena test.

Against this background a modified arena test was used at Elsenburg to test progeny from two Merino lines divergently selected for NLW (Cloete *et al.,* 2004; 2009) for potential line differences, genetic (co)variances for and among traits as well as genetic trends. Since the design involved a bidirectional genetic treatment, genetic trends were tested for divergence as well as symmetry of responses.

6.3 Materials and Methods

6.3.1 Experimental animals and husbandry

Merino lambs, born between 2001 to 2015 in a resource flock consisting of two divergently selected Merino lines at Elsenburg (Schoeman *et al.,* 2010), were assessed for their response to humans at the age of approximately 8 months. A ranking table that used principles suggested by Turner (1977) were used to select male and female replacements in the respective lines on maternal performance for NLW, where this selection resulted in the widely divergent lines from 1986 to present (Cloete & Scholtz, 1998; Cloete *et al.,* 2004; 2009; Scholtz *et al.,* 2010a). Ranking values were complemented and later on practically substituted by breeding values for NLW derived from a single-trait, repeated-records animal model used to analyse annual reproduction records. These lines were named as the High line (H line) for upward selection and the Low line (L line) for downward selection. Both the H line and L line were grazed in the same flock, but no genetic material was exchanged between these lines in the animals included. Between 1997 and 2002 the overall means for NLW were 1.16 in the H line and 0.78 in the L line (Cloete *et al.,* 2003c) and evident divergence in NLW responses was evident (Cloete *et al.,* 2004a; 2017).

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The test animals were born during the winter (June-July; Cloete & Scholtz, 1998) and were usually tested by the end of their birth years. At that stage the test animals were maintained in a mixed-sex flock and grazed a mixture of dryland or irrigated lucerne, oat fodder foggage and irrigated kikuyu, depending on seasonal availability and the prevailing climate within specific lambing seasons. During the test period they were grazed on 0.5 ha irrigated kikuyu pasture in the proximity, but outside visual range, relative to the test arena. After being tested, test animals returned to the flock and the pastures described previously.

6.3.2 Test procedure

An arena test (Murphy *et al.,* 1994; Kilgour & Szantar-Coddington, 1995) that was modified as described by Cloete *et al.* (2005b) was used to assess these animals over a 15-year period from 2001 to 2015. The arena used was measured at 10.6 m x 4.0 m and the floor was marked out in 18 rectangles of equal size that were numbered from 1 to 18. An adjoining pen at one end of and on the outside of the arena contained six to seven contemporaries of the individual test animal. This pen was separated from the arena by a split pole fence with spaces measuring 30 cm between the horizontal split poles, allowing visual contact between the pen and arena. A human operator was seated on a chair directly in front of this pen and on the inside of the test arena. The test animal was introduced into the arena by a second operator at the furthest point (10.6 m) from the seated human and was individually observed for 3 minutes by two recorders situated in a building overlooking the test arena. Every 15 seconds the presence of the animal in the marked rectangles was recorded. The behaviour of the sheep was described by the following derived traits: 1) the mean distance from the seated operator based on the distance from each square from the human, 2) the total number of boundaries between squares that were crossed by the test animal (as based on square the front left foot of the animal was situated in) at 15 s intervals (these crosses were used as an indication of the total distance travelled in the arena), 3) The number of high- and low-pitched bleats emitted by the animal, 4) the number of times the test animal urinated or defecated during the 3-minute test period.

As suggested by Murphy *et al.* (1994), the repeatability of similar measurements of temperament were fairly high (>0.55). Unfortunately, Murphy *et al.* (1994) did not partition the animal effects they observed in genetic and permanent environmental components. Against this background, the animals were only tested once.

6.3.3 Statistical appraisal

All counts were square root transformed to normalise the distributions prior to analysis after 0.5 was added to ensure a smaller difference between raw counts of 0 and 1 (Dickinson & Sanford, 2005). A five-trait animal model that included the single random effect of the animal (Gilmour *et al.,* 2015) was used to analyse the data recorded where the fixed effects included: selection line (H or L line), year of birth (2001 to 2014), sex (ram or ewe), age of dam (2 to 6+ years) and birth type (single or multiple). The five-trait analysis also allowed the estimation of genetic, residual and phenotypic correlations among traits. Estimated breeding values, as represented by animal solutions obtained from the output of a five-trait analysis that excluded selection line and its interactions with birth year, were used to assess possible

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divergence with reference to genetic differences between lines. The inclusion of selection line in this analysis would have corrected for genetic differences that accrued because of the selection imposed on the resource flock. Annual breeding values were regressed on birth year to assess genetic trends that resulted as correlated responses to the selection for NLW that were practiced. The pedigree file contained 8739 animals, which were the progeny of 335 sires and 1954 dams.

6.4 Results and Discussion

6.4.1 Descriptive statistics

The descriptive statistics for the data analysed are provided in Table 6.1. The count data were normally distributed after transformation. The coefficients of variation ranged from 26% for the number of crosses to 37% for the distance from the operator. We did not find any comparable results in the literature to relate these values to.

Table 6.1 Descriptive statistics for the average distance from the operator (DIST) and square root transformed counts for the number of crosses (CROSS), bleats (BLEAT), urinating events (URINE) and defecating events (DEFEC) after 0.5 was added (n=2291)

Effect and level	Trait				
	DIST	CROSS	BLEAT	URINE	DEFEC
Mean±s.d.	3.82±1.24	4.18±1.24	3.89±1.44	1.07±0.31	1.11±0.41
CV (%)	37.2	25.8	37.0	29.0	36.9
Range	1.07–9.20	0.71–9.19	0.71–8.34	0.71–2.55	0.71–2.35
Skewness	0.71	0.44	-0.46	-0.49	-0.83
Kurtosis	0.21	0.53	-0.14	0.23	0.50

6.4.2 Fixed effects

The fixed effects solutions for the effects of selection line and sex are provided in Table 6.2. The back-transformed means are discussed as they resemble the actual counts recorded, but the least squares means for the transformed data were tabulated. The H line lambs were less likely to urinate and defecate, more closely approached the seated human operated and would travel longer distances during the test than their L line contemporaries ($P<0.05$). The mean distance from the operator of L line lambs amounted to 6% further than that of their H line contemporaries. For the number of crosses in the L line, the back-transformed means amounted to 85% of those in the H line (15.4 vs 17.5). For urination and defecation events in L line progeny, the mean number exceeded that of their H line contemporaries by respectively 28% (back-transformed means of 0.82 vs. 0.64) and 19% (back-transformed means of 0.87 vs. 0.73).

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Table 6.2 Depicting the effect of selection line on the average distance from the operator (DIST), counts for the number of crosses (CROSS), bleats (BLEAT), urinating events (URINE) and defecating events (DEFEC) in Merino weaners (± s.e.)

Effect and level	Trait				
	DIST	CROSS	BLEAT	URINE	DEFEC
Line	**	**	0.30	**	*
H line	3.77±0.04	4.24±0.03	3.83±0.04	1.07±0.01	1.11±0.01
L line	3.99±0.10	3.98±0.08	3.90±0.08	1.15±0.02	1.17±0.02
Sex	0.58	0.157	**	**	**
Ram	3.90±0.06	4.08±0.05	3.66±0.05	1.13±0.01	1.11±0.02
Ewe	3.86±0.06	4.15±0.05	4.07±0.05	1.09±0.01	1.17±0.02

* - P<0.05; ** P<0.01; Actual significance for P>0.05

When socially isolated, sheep become more vocally active and show behavioural changes. There is an increase in behavioural withdrawal and if the isolation is prolonged there may be a reduced intake of food and water (Nowak *et al.*, 2008). Flocks selected for rearing ability was found to move around the arena less and bleated less than the unselected flock which could indicate that the selected flock were less agitated when placed in an arena when no flock-mates are present (Kilgour, 1998). This result seem to be in contrast with the findings of the present study, as H line weaners travelled longer distances than their L line counterparts. Beausoleil *et al.* (2008; 2012) found similar results in sheep that were selectively bred for their locomotor activity and behavioural responses to isolation (see initial paper by Murphy *et al.* 1994 for more background), namely More Active (MA) and Less Active (LA) sheep. MA sheep were similar to the H line as they were less fearful, as they spent more time near the operator and approached them closer and travelled more for the duration of the test than their LA contemporaries which were similar to the L line. As concluded in this study, the authors concluded that MA sheep became habituated sooner to the test environment than the LA contemporaries. Differences between extensively and intensively reared sheep has also been seen. Intensively reared sheep showed more locomotor activity than extensively reared sheep (Goddard *et al.*, 2000; Hazard *et al.,* 2016), and intensively reared animals also emitted more high-pitched bleats than extensively reared animals (Hazard *et al.,* 2016). There were significant within flock effects suggesting that if a flock had a high average flight speed it would not necessarily also have a high average agitation score as reflected by increased activity in the arena (Dodd *et al.*, 2014).

Sex differences were observed, as ewe lambs were more likely to bleat and defecate than ram lambs, but less likely to urinate (P<0.05; Table 6.2). Previously, ewe lambs were found to be more active than ram lambs, but ram lambs spent more time near a motionless human operator than ewe lambs, even though the ewe lambs had a strong attraction to the flock mates (Boissy *et al.*, 2005). In the present study, the higher number of bleats emitted by ewes and the higher defecation rate of ewes may be associated with a similar attraction to their flock mates. Ewe lambs were more reactive than ram lambs in the agitation test (Dodd *et al.*, 2014), while ewes were more fearful to people and attempted to escape more often than rams (Vandenheede & Bouissou, 1993; Boissy *et al.*, 2005). However, Boissy *et al.* (2005) suggested that

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the ewes were just more socially motivated than rams. In animals selected for rearing ability it was seen that behaviour of rams and ewes differed from ewes and rams of an unselected flock. Between the flock selected for rearing ability and the unselected flock the total distance travelled was consistently different followed by the number of bleats (Kilgour, 1998). The rams of the two flocks differed significantly from each other at 6 months of age, this was however not seen in both 6- and 12-month measurements in ewes of both flocks. It was also concluded that bleats may be a more useful indicator in ewes than in rams. Older lambs were less reactive in the agitation test than younger lambs.

Finally, arena traits were found to be independent of birth type and dam age (P>0.10). The age of the dam and birth type similarly did not influence the expression of emotive traits in study by Boissy *et al.* (2005). A lack of birth type effects on arena traits was also reported by Wolf *et al.* (2008). Year affected all arena traits (P<0.01). Significant year effects were not tabulated bur were included in the genetic analysis for the variation they controlled. Lambe *et al.* (2001) and Wolf *et al.* (2008) found no relationship between the live weight of the sheep and its behaviour.

6.4.3 Genetic parameters

The distance lambs maintained from the seated operator was lowly heritable (0.08±0.03; Table 6.3). Wolf *et al.* (2008) also suggested that traits associated with the distance from the human in the arena are lowly heritable, estimates ranging from 0.02 to 0.22. Boissy *et al.* (2005) reported a moderate heritability (0.23) for reactivity towards the human operator. The number of crosses and bleats were moderately to highly heritable at respectively 0.22±0.04 and 0.35±0.05. These estimates are consistent with previous results reported by Wolf *et al.* (2008), namely 0.29 for boundaries crossed and 0.39 for number of bleats. The heritability of the number of urinating events was smaller in magnitude at 0.13±0.03. The number of defecating events was lowly heritable at 0.04±0.02. Significant genetic correlations suggested that animals maintaining a larger distance from the operator crossed more rectangles, were more likely to urinate and tended to have more defecation events (Table 3). Strong genetic correlations were estimated for vocalisation activity and locomotion behaviour (Hazard *et al.*, 2016). Heritabilities for vocalisations were high (0.46-0.53 for high-pitched bleats; 0.35-0.43 for low-pitched bleats), locomotion had moderate heritability (0.29-0.31) and reactivity towards humans had low heritability (0.12-0.17), with low genetic correlations between vocalisations and locomotion and high genetic correlations between locomotion and proximity to motionless human (Hazard *et al.,* 2016).

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Table 6.3 Phenotypic variance (σ^2_P) and correlations between the average distance from the operator (DIST) counts for the number of crosses (CROSS), bleats (BLEAT), urinating events (URINE) and defecating events (DEFEC)

Component and trait	Trait				
	DIST	CROSS	BLEAT	URINE	DEFEC
σ^2_P	2.415	1.462	1.894	0.0918	0.1559
(Co)variance ratios*					
DIST	***0.08±0.03***	0.39±0.18	0.26±0.17	0.46±0.23	0.35±0.22
CROSS	0.20±0.02	***0.22±0.04***	0.05±0.13	0.22±0.17	-0.14±0.24
BLEAT	0.33±0.02	0.27±0.02	***0.35±0.05***	0.12±0.15	0.26±0.23
URINE	0.01±0.02	0.01±0.03	0.04±0.02	***0.13±0.03***	0.37±0.24
DEFEC	0.10±0.02	-0.02±0.02	0.02±0.02	0.23±0.02	***0.04±0.02***

* Heritability in bold on the diagonal, genetic correlations above the diagonal and phenotypic correlations below the diagonal

Animals with more urination events tended to also exhibit more defecation events. Dodd *et al.* (2014) found that flight speed and agitation had low to moderate heritability estimates of 0.11±0.02 and 0.20±0.02 respectively. They also concluded that the behavioural tests were poorly correlated phenotypically and genetically with each other. Both flight speed and agitation increased with the weight of the lamb as heavier lambs were more reactive, which was in contrast to findings of Pajor *et al.* (2008) who reported that heavier lambs were calmer. Phenotypic correlations in the present study were in the same direction as genetic correlations but mostly smaller in magnitude. The majority of the estimates for measured behaviours indicate that they were moderate to high repeatability (Murphy *et al.*, 1994; Kilgour & Szantar-Coddington, 1995; Kilgour, 1998; Wolf *et al.*, 2008). This result indicates that the measure of vocalisations, locomotion and human-related behaviours in arena tests are generally replicable across time in follow-up events, even though habituation may occur. Habituation to the test environment and maintenance of lowered response to the test environment for periods up to one year have been reported (Kilgour & Szantar-Coddington, 1995; Kilgour, 1998; Goddard *et al.*, 2000; Wolf *et al.*, 2008).

6.4.4 Genetic line differences and trends

Phenotypic differences between the H and L lines were reported in Table 6.2. This section assessed differences at the genetic level as reflected by five-trait breeding values that were obtained from the analyses conducted during the course of the study. The selection line averages for the breeding values (Table 6.4) were lower in H line for the average distance from the human operator, number of bleats, urination events and defecation events when compared to their L line contemporaries. However, the average breeding values for the number of crosses were higher in the L line. These results complemented those discussed previously when the phenotypic line differences were considered.

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Table 6.4 Overall means (±s.e.) for estimated breeding values for the arena behaviour traits derived from the multiple trait analysis not incorporating selection line as a fixed effect

Trait	Selection line	
	H Line	L Line
Average distance from human (m)	0.034 ± 0.004	0.259 ± 0.009
Transformed counts		
Crosses (#)	0.271 ± 0.006	0.012 ± 0.015
Bleats (#)	-0.118 ± 0.009	-0.004 ± 0.020
Urination events (#)	-0.024 ± 0.001	0.052 ± 0.003
Defecation events (#)	-0.027 ± 0.001	0.019 ± 0.002

Selection line differences were significant (P<0.01) for all arena traits

It now remains to be seen whether the genotypic line differences from Table 6.4 were stable across time or whether genetic changes took place during the 15-year period from 2001-2015. Most of the derived regressions of averaged breeding values on birth year had significant slopes (p<0.05) (Table 6.5). The exceptions to this rule were the number of crosses and number of bleats recorded in both H and L line lambs. The regression coefficients between lines for all traits observed differed.

Table 6.5 Details of linear regression equations depicting genetic change as reflected by the regression of averaged predicted breeding values on years of behavioural traits that were measured on lambs

Trait and line	Slope (b±s.e.)	Intercept (a±s.e.)	R^2	r
Average distance from operator				
H line	-0.0041±0.0018	0.0627±0.0151	0.28	0.53*
L Line	0.0250±0.0060	0.0743±0.0490	0.57	0.76*
Number of crosses				
H line	0.0030±0.0021	0.2502±0.0170	0.14	0.38
L Line	-0.0016±0.0046	-0.0231±0.0376	0.01	0.10
Number of bleats				
H Line	-0.0044±0.0044	-0.0869±0.0358	0.07	0.27
L Line	0.0112±0.0121	-0.0826±0.0996	0.06	0.25
Number of times urinated				
H Line	-0.0040±0.0012	0.0039±0.0101	0.45	0.67*
L Line	0.0058±0.0015	0.0110±0.0123	0.54	0.73*
Number of times defecated				
H Line	-0.0014±0.0006	-0.0172±0.0046	0.32	0.56*
L Line	0.0022±0.0005	0.0037±0.0040	0.60	0.77*

* Denotes significant (p<0.05) regressions

Trends in the H line were negative for average distance from the operator and the number of urination and defecation events. As for the intercepts, only number of bleats and number of defecation events were negative. Similar to the regression coefficient the intercept for the L line is only negative for number of crosses. The breeding values for the mean distance from the operator (Figure 6.2) in the H line was reduced at -0.11% per annum, while in the L line the breeding values increased at 0.65% pe annum. As for the annual breeding values for the number of crosses (Figure 6.3) no genetic changes seemed to

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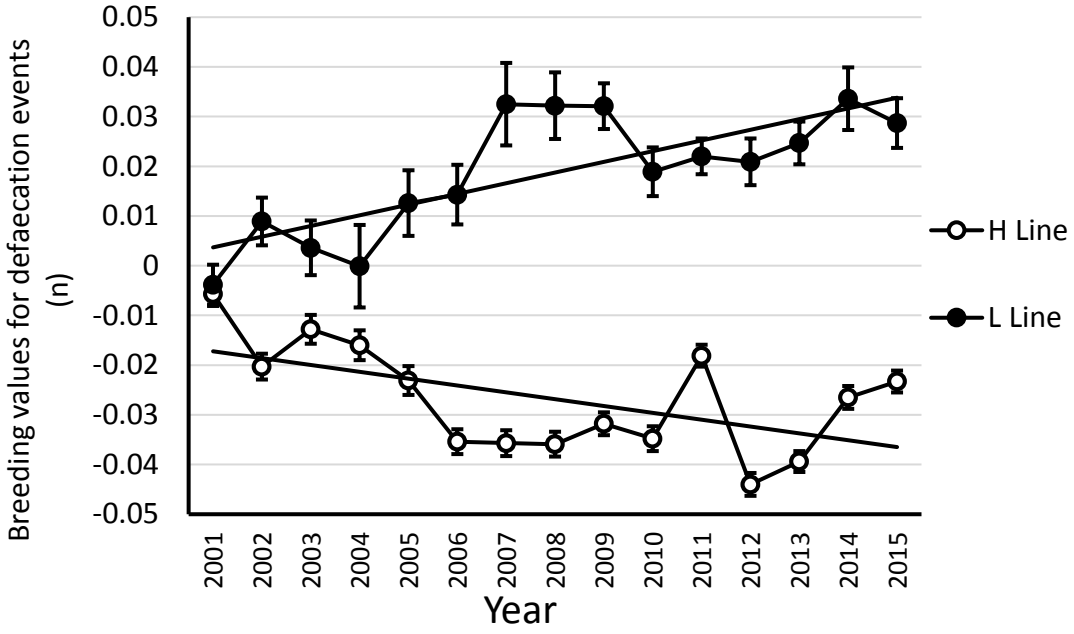
accrue in either line. However, breeding values in the high line ranged from 0.25 to 0.33, while L line breeding values oscillated around zero. Averaged annual breeding values for both lines for the number of bleats were quite stable over the years (Figure 6.4) but were distinctly more variable in the L Line. For urination (Figure 6.5) and defection (Figure 6.1) events the annual breeding values in the H line exhibited a downward trend at respectively -0.37 and -0.13% of the respective overall means. Corresponding genetic trends in the L line were upward, increasing at respectively 0.54 and 0.20% of the respective overall means.

Figure 6.1 Means (± s.e.) for annual estimated breeding values for number of defecation events of



the H and L line lambs. Breeding values are presented on the square root transformed scale.

As seen by both the average breeding values and linear regression equations, the H line approached the human operator closer, urinated and defecated at a lower frequency, and bleated somewhat less than their L line contemporaries. However, L line lambs covered a smaller distance for the duration of the test than the H line lamb, both at phenotypic (Table 6.2) and genetic (Table 6.4) levels. Some of these behavioural repertoires in the H line suggested lower levels of stress as discussed previously. For instance, defecation and/or urination are widely accepted as responses to stressful conditions in small laboratory animals (Archer, 1973).

These behavioural responses in the H line are additional desirable correlated responses to selection for NLW on animal welfare level. These changes, along with an improved lamb survival rate (Cloete et al., 2009), a reduced susceptibility to flystrike (Scholtz et al., 2010a) and a reduced crutching time (Scholtz et al., 2012) in the H line are all considered as beneficial to animal welfare.

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6.5 Conclusions

The research has shown that some behavioural differences in sheep are heritable. From an ethical point of view, the genetic adaptation of farm animals to exhibit lower levels of fear during routine farm operation is highly desirable. The favourable correlated responses to selection for NLW in the reaction of H line animals towards humans needs to be verified by the estimation of genetic correlations with NLW before possible inclusion in selection programmes in future.

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Chapter 7
General conclusions and future recommendations

General conclusions are first provided per chapter, before being consolidated to form recommendations and suggestions for further research.

Chapter 3: When birth weight, lamb survival, and perinatal behaviour were assessed it was concluded that that the suckling behaviour and the time it takes the lamb to progress to suckling was independent of selection line and sex but was influenced by the age of the dam. This would suggest that this crucial behaviour of the lamb was reliant on the experience of the ewe that one would naturally attribute to increasing dam age. With experience and age this progression to suckling becomes shorter which was also supported by this trait being affected by cooperation of the dam. As the age of the dam increased, ewes performed less aversive behaviour repertoires that could interfere with the lambs' attempts at suckling. Birth weight was influenced by the selection line, the sex of the lamb, birth type and the age of the dam. The slightly heavier birth weight in the H Line lambs might have contributed to these lambs having a higher survival rate than their L line contemporaries. Single lambs progressed to suckling quicker and had heavier birth weights. Second-born multiples experienced shorter lengths of parturitions than either singles or first-born multiples irrespective of selection line, but overall H line lambs experienced shorter births than their L line contemporaries. This difference was particularly evident in first multiple lambs.

It was evident that some behaviour traits were heritable at the direct and/or maternal levels. A major outcome of this chapter was the estimation of genetic correlations of behaviour traits with lamb survival. Given the importance of lamb survival to output, as well as to animal welfare, as well as the lack of clear recommendations with regard to the genetic improvement of lamb survival, this was an important outcome. Line differences, genetic parameters as well as a maternal genetic trend for latency of parturition in the H line all combined to establish that it was possible to genetically improve lamb survival. Such improvements would furthermore be associated with behaviour repertoires conducive to the rearing of lambs. These results make an important contribution to the current knowledge on this topic.

Chapter 4: When the number of lambs weaned per ewe mated (NLW) and perinatal behaviour of ewes were assessed, it was evident that the time ewes remained on the birth site was not affected by the selection line, although older ewes and ewes caring for multiples remained on their birth sites for longer periods. This indicates that experience of the ewe determined the period she remained at the birth site. Ewes that remained on the birth site for longer periods exhibited higher maternal cooperation scores which in turn facilitated a quicker progression of the lamb from birth to suckling. As the age of the dam increased so did the NLW. NLW also differed between selection lines, where H line ewes had a higher NLW than their L line contemporaries. In accordance with results from Chapter 3, length of parturition decreased in smaller litters and was also shorter in H line ewe compared to L line contemporaries. Both Chapters 3 and 4 demonstrated an improved maternal cooperation with first suckling attempts of progeny in H line ewes.

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A key output was that ewe behaviour traits were heritable and that length of parturition as well as ewe cooperation score were favourably correlated with NLW on the genetic level. It is presently debated as to whether composite trait selection for NLW or total weight of lamb weaned per ewe mated or selection based on an economic index of all the component traits of reproduction would be best. However, very low levels of additive variation for ewe rearing ability (as a proxy for lamb survival) complicate reasoning with reference to index selection for component traits of NLW. These results therefore contribute to our present understanding of the genetic improvement of ovine reproduction.

Chapter 5: This chapter allowed responses to divergent selection for NLW in the H and L lines to be assessed in the absence of possible dam effects, as all lambs were reared by randomly chosen surrogate dams. Year to year variation in embryo yield per donor were more evident in the L line than in the H line, indicating that H line ewes were more likely to be consistent in the number of embryos harvested. Production traits (birth weight, birth coat score and weaning weight) were not affected by the selection line, but the survival of H line lambs was improved when compared to their L line contemporaries. This result indicated that H line lambs were inherently capable of a higher survival rate, even when reared by randomly allocated surrogate dams. This line difference was also evident in Chapter 3, but there it was confounded with maternal effects of the lambs being born within lines. Yearling weight and wrinkle score were improved in H line progeny relative to L line contemporaries, as was also suggested in previous within line genetic analyses. Wool traits remained independent of selection line, except for fibre diameter where finer diameters were observed in the H line. Improved reproduction was observed in the H line embryo transfer progeny. However, it was impossible to relate the improved survival of embryo transfer lambs conclusively to behavioural differences, as was feasible in Chapter 3. It seems like other mechanisms than the observed behaviour traits were instrumental in the line difference for lamb survival in embryo transfer progeny.

Chapter 6: H line lambs seemed to cope better with the stress of the arena test than their L line contemporaries, as reflected by a closer proximity to the seated human, as well as fewer urination and defecation events. These results indicated that the H line lambs were not as conflicted in their motivation to be with their flock mates when exposed to an immobile human seated between the test animal and its flock mates. These differences were also found between ewe and ram lambs as ewes were more active for the duration of the test than ram lambs. The number of urination and defecation events, as well as average distance from the seated human all had low but significant levels of additive genetic variation. Moderate heritability estimates were derived for vocalisations and the distance travelled in the test. Vocalisations are considered as a good indication of the reaction of animals to the novel environment. Animals that maintained a larger distance from the human were more likely to urinate and defecate. As urination and defecation are considered to be indicators of stress experienced by animals, these genetic correlations suggested that animals with higher levels of fear, keeping them further from their flock mates, would vent their unease with the situation in this way. Genetic trends indicated that, over the 15 years of

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recording, H line animals maintained their distance from the seated human, while L line animals increased it. H line progeny also became less likely to urinate and defecate, while these indicators of stress became more prevalent in the L line.

The significant genetic variation in arena behaviour, as well as genetic trends in the H and L lines all added to an improved understanding of behavioural attributes in response to human presence and flock isolation. These results now allow analysts to consider the role that the arena test or similar tests may play in sheep breeding for improved welfare and a reduced level of fear to humans during routine husbandry operations.

Recommendations: Several recommendations can be made from the foregoing synthesis of key findings from the study, namely:

Research on key production and welfare traits like lamb survival should continue. Based on differences between the H and L line for lamb survival (Chapters 3 and 5), as well as differential survival patterns for multiples, future studies may have to investigate the survival of singles and multiples as separate traits to ascertain that the genetic architecture is the same for both singles and multiples.

Maternal behavioural scores at lambmarking have not yet been studied extensively in South Africa. Ease of recording and evidence of favourable (although moderate) genetic correlations with lamb output reasons in favour of collection of data to assess this trait in the local small stock population.

Studies on improving NLW genetically should continue. Taking a cue from international trends, it would be appropriate to assess composite trait selection in tandem with component trait selection to better understand the possibilities that the different chains of thought offer to sheep breeding at the national level.

The study reaffirmed the value of the resource flock used to sheep breeding in South Africa. The flock, together with other resources under institutional control will play an important role in the establishment of a reference population for genomic selection in the South African ovine genetic resource. Studies towards the establishment of such a population should continue.

Tests like the arena test may play a role in promoting welfare on the flock level. There are clear indications that research on this test, as well as alternative indicators of the reaction of animals to human presence and flock isolation should continue. A first objective should be to derive genetic correlations of arena behaviour with traits of economic importance. Traits in several complexes, such as growth, wool traits, resistance as well as reproduction should be obvious targets for further study.

The genetic architecture of complex traits such as ewe, individual lamb behaviour, composite reproduction traits as well as other hard-to-measure traits could be targeted to great effect in future academic studies. With the number of genotyped animals in the local small stock genetic resource set to increase in future, it should be a priority to assess SNP-genotypes at the genome-wide level with known phenotypes for such traits. With sheep in institutional resource flocks often having more phenotypes than industry animals it may be worthwhile to extend some behavioural tests to other flocks, should the utility of such tests be illustrated in future studies in those flocks where it is routinely practiced at present.

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Potential genetic markers for welfare traits would be a great advantage, given the difficulty associated with acquiring welfare data on a national level.

Clearly there is a need for defining other potential indicators associated with the welfare of free ranging sheep. Apart from the arena test, there are other international tests that could be adapted for assessment in local ovine resource flocks. Such tests may be considered separate from, or in combination with the arena test. Test involving the isolation box, docility tests, flight speed, response of animals to handling in sheepyards or in open spaces could typically be considered in future studies.

Finally, a search should be undertaken to potential biological markers that could be used as a proxy for typical welfare traits. The H and L lines were in the past demonstrated to be different in their reaction to stress at the level of the hipothalamus-pituitary-adrenal axis. Non-invasive sampling, such as for wool cortisol, could potentially play a role in this respect. Further studies on the potential of such biomarkers will be of great value.

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Addendum

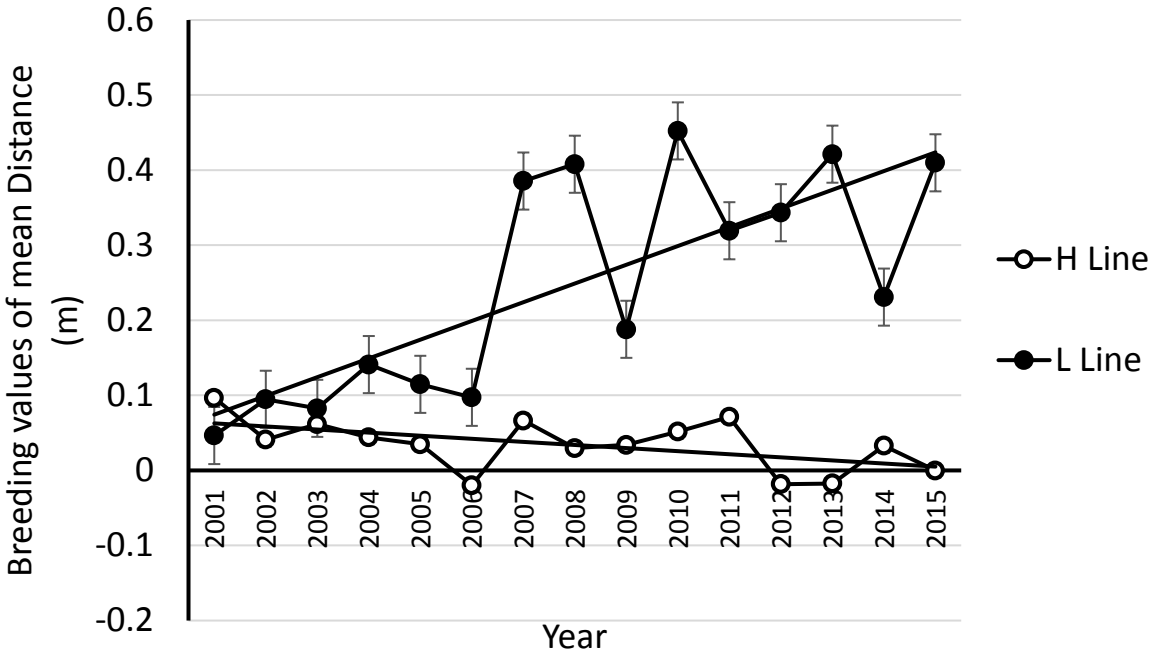

Figure 6.2 Genetic trend (± s.e.) for mean distance from operator of the H and L line lambs. Breeding values are presented on the observed scale.

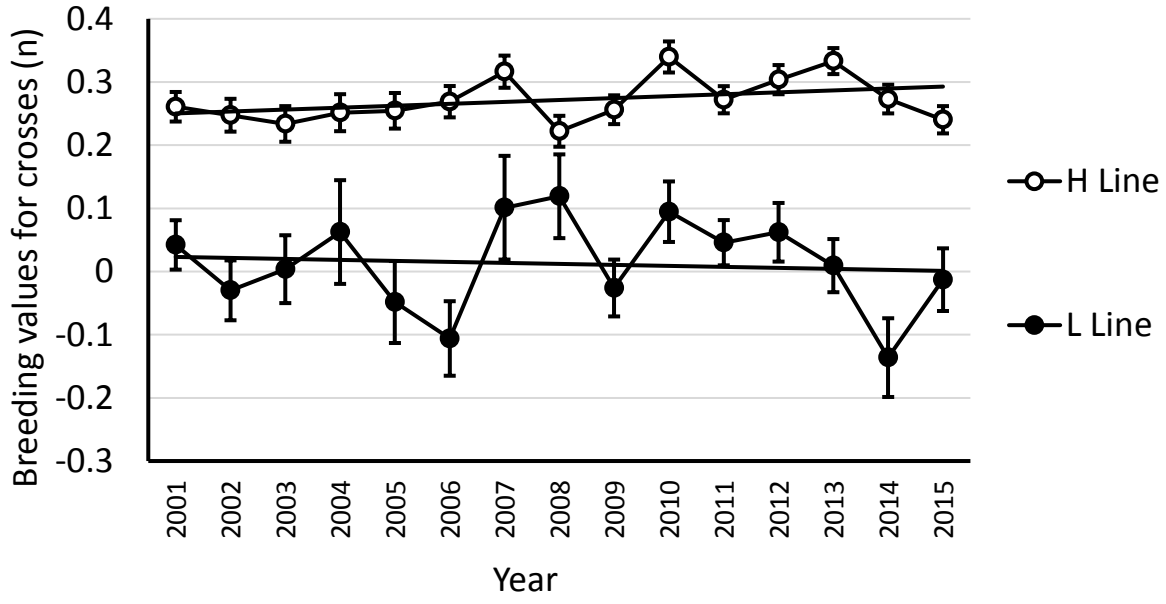

Figure 6.3 Means (± s.e.) for annual estimated breeding values for mean number of crosses of the H and L line lambs. Breeding values are presented on the square root transformed scale

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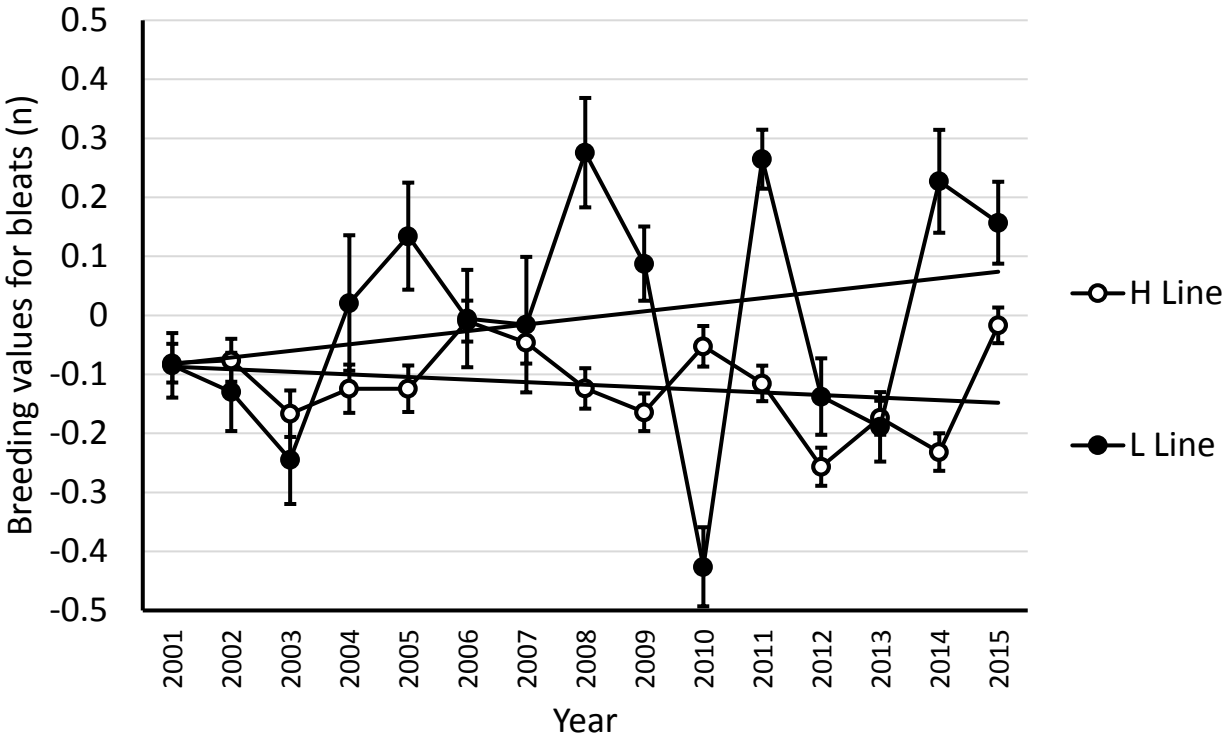

Figure 6.4 Means (± s.e.) for annual estimated breeding values for number of bleats of the H and L line lambs. Breeding values are presented on the square root transformed scale

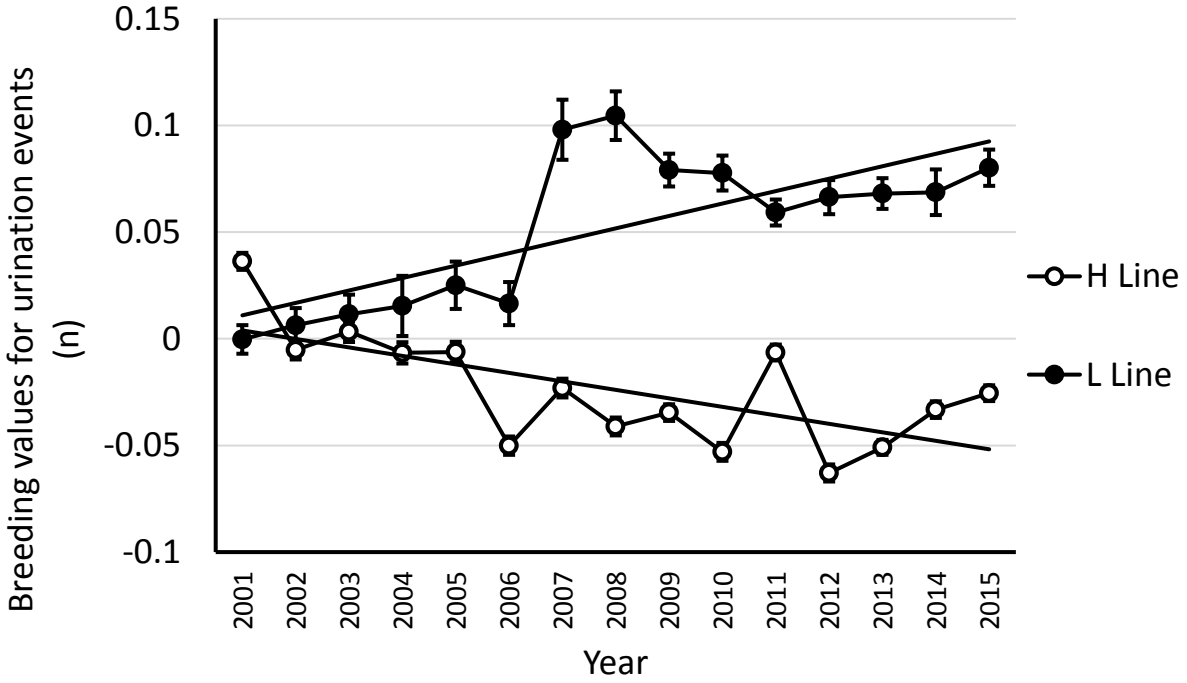

Figure 6.5 Means (± s.e.) for annual estimated breeding values for number of urination events of the H and L line lambs. Breeding values are presented on the square root transformed scale.

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